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Melanoma

Various aspects of presentation, treatment and outcome

2

Rijks*universiteit* Groningen

Melanoma

Various aspects of presentation, treatment and outcome

Proefschrift

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4

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6

For my Parents

8

Contents

Chapter I

❑   Introduction and aims of the thesis

❑ **Incidence**

Cutaneous melanomas (CM) are tumors with an unpredictable biological behavior. The incidence of cutaneous melanoma is still increasing worldwide, and despite significant advances in prevention, early detection and treatment, the mortality rate continues to increase steadily in certain countries.[1-3] The current incidence rates vary dramatically throughout the world, ranging from as low as 0.2 per 100,000 in China to as high as 40.5 per 100,000 among males in Australia.[4] Mortality rates are more than fivefold lower than incidence rates and appear to be plateauing and declining in some groups. The highest mortality rate is reported from New Zealand and is estimated to be 5.3 per 100,000.[4] Currently the incidence rate and mortality rate in the Netherlands are respectively 12.6 and 3.1 per 100,000 people, with 2100 new cases of melanoma being diagnosed in 1998.[5] Because of early detection and treatment, melanomas are however being diagnosed at an earlier stage compared to in the past. This has led to an increase in the incidence of thin lesions (<1.00 mm Breslow thickness) among young individuals (those under the age of 65), with continuing increases in thick melanomas for males over the age of 65.[1] This is one of the reasons that the mortality rate has recently stabilized. However one in four patients will develop loco-regional recurrence and one in five patients will still develop distant metastases.[6]

❑ **Prognostic Factors and Staging**

The overall prognosis for patients who have localized melanoma without any nodal or distant metastases is good. The 5-year survival rates for patients with Stage I and II melanomas are respectively 93% and 68%.[7] Several features of the primary tumor and lymphatic metastases have been examined as potential prognostic factors predictive for melanoma metastases. The analysis of the American Joint Committee on Cancer (AJCC) database on melanoma has confirmed that the risk of melanoma metastases increases with tumor thickness, the presence of ulceration, increasing age, anatomic site and nodal status.[8]

12

Breslow tumor thickness

Breslow microstaging determines the thickness of the lesion, in millimeters, using an ocular micrometer to measure its vertical height. This was first described in 1970 by Breslow in which he established a correlation between tumor thickness and survival.[9] The risk of melanoma local recurrence increases with tumor thickness (Table 1).

Thickness (mm)	n	Local recurrence (%)	In transit metastases (%)	Regional nodal recurrence (%)	Distant metastases (%)
1.00-2.0	445 (60%)	2.3	3.6	9.2	14.4
2.01-3.0	215 (29%)	4.2	8.4	15.8	27.9
3.01-4.0	77 (10%)	11.7	16.9	29.9	44.2
Total	737 (100%)	3.8	6.4	13.3	21.4

Table 1. Frequency of subgroups of thickness and incidence of local recurrence and other metastases.[6]

Tumor ulceration

Another factor associated with a greater risk for local recurrence is the presence of ulceration in the primary melanoma. When compared to non-ulcerated lesions, ulcerated melanomas are believed to represent a more biologically aggressive form of the disease that is associated with a worse prognosis. The presence /absence of ulceration was determined to be the second most powerful independent predictor of survival among factors analyzed by the American Joint Committee on Cancer (AJCC) behind Breslow thickness.[8]

Tumor mitotic rate

Recently researchers at the Sydney Melanoma Unit analyzed 3661 patients with regard to prognostic indicators and determined that tumor mitotic rate, and not

ulceration, is the second most powerful prognostic indicator behind tumor thickness in regard to overall survival. If confirmed by studies from other centers, it has the potential to further improve the accuracy of melanoma staging.[10]

MD Anderson Staging System

The MD Anderson staging system is a four-stage system designed specifically to address the subset of patients with local, in transit, or satellite recurrences who were suitable candidates for isolated limb perfusion (Table 2). This staging system was used in chapters three and four, but is hardly longer used today.

2002 AJCC TNM Melanoma Staging System

In 2002 the new TNM AJCC staging system (Table 3 + 4) was completed and introduced as the new standard in melanoma staging.[7] This staging was revised from the previous staging system in 1997. The major changes applied include incorporating the tumor thickness and ulceration directly into the T(umor) category and defining the N(ode) category by the number of metastatic nodes and not by the gross dimensions of the affected nodes (Table 3). This staging system was used in chapters five, six and seven.

Stage	Description
IA	Primary tumor only
IB	Primary excised
IC	Multiple primaries
II	Local recurrence (within 3cm)
IIIA	In-transit recurrence
IIIB	Regional nodal recurrence
IIIC	In-transit and regional nodal recurrence
IVA	Distant cutaneous metastases
IVB	Distant visceral metastases

Table 2. *The MD Anderson staging system*

T Classification	Tumor Thickness (mm)	Ulceration status
T1	≤1.0	a. without ulceration and level II/III
		b. with ulceration or level IV/V
T2	1.01-2.0	a. without ulceration
		b. with ulceration
T3	2.01-4.0	a. without ulceration
		b. with ulceration
T4	> 4.0	a. without ulceration
		b. with ulceration
N Classification	**No. of metastatic nodes**	**Nodal metastatic mass**
N1	1 node	a. micrometastasis
		b. macrometastases
N2	2-3 nodes	a. micrometastasis
		b. macrometastases
		c. satellite(s)/in transit metastases without metastatic nodes
N3	> 4 nodes, or in-transit metastases with metastatic nodes	
M Classification	**Site**	**Serum LDH**
M1a	Distant skin, subcutaneous or nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases	Normal
	Any distant metastasis	Elevated

Table 3. The 2002 TNM AJCC melanoma staging system.[7]

❑ Aims of the Thesis

Over the past 35 years over two and a half thousand patients were treated for a primary cutaneous melanoma at the Groningen University Medical Center (GUMC). The GUMC is a referral center for over 2 million people located in the northern part of the Netherlands. Besides treating the usual cases of melanoma, an extensive experience in treating locally advanced melanoma has been developed such as hyperthermic isolated limb perfusion (ILP) and unusual cases of melanoma. Data from all of these patients has been collected and stored in a melanoma database. This database contains more than one-hundred patient, tumor and treatment characteristics. This thesis is based upon these patients and is divided in to two parts (A, B)

❑ Part A Hyperthermic Isolated Limb Perfusion

Hyperthermic isolated limb perfusion was introduced in 1958 by Creech and Krementz.[11] Six years later, in 1964 the first isolated limb perfusion for melanoma was performed by Oldhoff at the Groningen University Medical Center.[12] The main advantage of this treatment modality is that a high dose of cytostatic drug can be delivered locoregionally without producing systemic toxicity. The indications for this treatment are inoperable local recurrence and in-transit metastases. Usage of this therapy in the adjuvant setting is discouraged since no benefit was seen in the multi-center EORTC trial.[13] **In chapter 2** a review of the history, indications, technique and refinement of the technique over the years is described.

Traditionally the cytostatic drug used in the treatment of ILP in melanoma is melphalan (L-phenylalanine mustard, GlaxoSmithKline, London, England) and recently since the start of the nineties tumor necrosis factor α (TNFα; Boerhinger-Ingelheim GmbH, Vienna, Austria) has been added to the regimen for bulky disease. A variety of other antineoplastic agents besides melphalan have been used during the last forty years in hyperthermic isolated limb perfusion such as darcarbazide (DTIC), actinomycin-D, thiotepa, mitomycine-C, doxorubicin and more recently cisplatin.[14]

	Clinical Staging				Pathological Staging		
0	Tis	N0	M0	**0**	Tis	N0	M0
IA	T1a	N0	M0	**IA**	T1a	N0	M0
IB	T1b/T2a	N0	M0	**IB**	T1b/T2a	N0	M0
IIA	T2b/T3a	N0	M0	**IIA**	T2b/T3a	N0	M0
IIB	T3b/T4a	N0	M0	**IIB**	T3b/T4a	N0	M0
IIC	T4b	N0	M0	**IIC**	T4b	N0	M0
III	Any T	Any N	M0	**IIIA**	T1-4a	N1a/N2a	M0
				IIIB	T1-4b	N1a/N2a	M0
					T1-4a	N1b/N2b/N2c	M0
				IIIC	T1-4b	N1b/N2b/N2c	M0
					Any T	N3	M0
IV	Any T	Any N	Any M	**IV**	Any T	Any N	Any M

Table 4. *2002 TNM AJCC Stage groupings for cutaneous melanoma*

The majority of the cytostatic agents were or ineffective, or the duration of response quite limited, or when a drug showed effectiveness the local toxicity hampered the further application.

17

In **chapter 3** a derivative of cisplatin, carboplatin was used in a feasibility study with regard to effectiveness and toxicity. Since the introduction of the pleiotrophic cytokine TNF α perfusionists have become more aware of the leakage of the TNFα to the systemic circulation. The most serious complication after TNFα perfusion is the systemic inflammatory response syndrome (SIRS) accompanied by fever, rise in cardiac output, fall in systemic vascular resistance and the need for fluid resuscitation and inotropes. If leakage exceeded the 2% limit during perfusion, there was less exposure of the tumor bearing limb to TNFα and an increased exposure of the patient systemic circulation to TNFα, resulting in more systemic side effects.[15] It is therefore imperative to have an effective systemic leakage monitoring system. In **chapter 4** our monitoring system which is based on continuous external monitoring with ^{131}I-albumin as the main isotope was studied for its effectiveness in reducing the amount of patients with excessive leakage.

❑   **Part B   Uncommon aspects of melanoma**

In this part of the thesis certain uncommon aspects of melanoma are described.

Pregnancy

Melanoma is often diagnosed in young adults and a significant number of these patients are women in their reproductive phase of life. The real incidence of melanoma during pregnancy is unknown. Smith and Randall reported in 1969 an incidence of 2.8 per 1,000 deliveries.[16] The influence of pregnancy on the prognosis of melanoma has been a controversy ever since 1951 when Pack and Scharnagel reported ten pregnant patients with malignant melanomas of which five died within 30 months after diagnosis.[17] Since then numerous case reports have also been presented showing a worse prognosis compared to non-pregnant women and the dilemmas in treatment policies.[18,19] In **chapter 5** the long term effect of pregnancy and early stage melanoma was studied for the patients who were pregnant and treated for a melanoma at the GUMC.

Childhood and Juvenile melanoma

Melanoma before puberty is rare, but the incidence increases steadily thereafter in each decade. The steepest trends of increasing incidence are in those aged over 65 and the lowest trends are in the younger age groups. Approximately 1 to 4% of all new melanoma cases occur in patients under the age of 20 years and only 0.3% among those younger than 14 years.[20-22] The highest rate is reported from Australia where 0.3% to 0.9% of the patients are under the age of 15.[23] In **chapter 6** we analyzed the childhood and juvenile melanomas treated at our institution with a special focus on differences in clinical characteristics, prognostic factors, disease free and overall survival in comparison with those of adults.

Brain metastases from Head and Neck melanoma

Cutaneous melanoma of the head and neck region comprise 10-20% of all melanoma.[22,24,25] It has been reported that patients with head and neck melanoma have a worse prognosis than those with extremity or axial lesions, with an overall 5-year survival of approximately 65%.[26] However, if local recurrence develops the 5-year survival drops to 36%.[27] Melanoma represents the third most common cause for the development of brain metastases, after primary tumors of the lung and breast.[28,29] The presence of brain metastases carries a worse prognosis in patients with metastatic melanoma and is one of the most common causes of death of this disease.[30] The question has been risen as to if there is a difference in incidence in brain metastases between head and neck cutaneous melanoma and extremity/truncal melanoma and which risk factors are responsible for this difference. In **chapter 7** we studied the patients with head and neck melanoma treated at the GUMC and which developed brain metastases in order to identify the risk factors associated with this devastating complication so that patients at highest risk can be monitored more closely and possibly treated earlier to improve the survival and quality of life.

In summary, the aims of this thesis are:

- To investigate the experience of isolated limb perfusion in the treatment of cancer

- To define the role of a new cytostatic agent carboplatin in the treatment of melanoma
- To evaluate the leakage of cytostatic agents during isolated limb perfusion
- To study the aspects of pregnancy in the treatment of melanoma
- To analyze the differences of childhood/juvenile melanoma in comparison to melanoma in adults
- To define the incidence and risk factors of brain metastasis in head and neck melanoma

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24

Chapter II

❑ Experience with isolated limb perfusion

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❑	**Introduction**

Locally advanced melanoma or sarcoma of the limb was in the past surgically treated by an amputation of the affected limb. The majority of the patients died from their disease despite an amputation. This lead Klopp at the end of the forties to the idea to deliver a cytostatic agent, nitrogen mustard, direct to the tumor bearing limb through an intra-arterial injection, as a limb saving cancer treatment.[1] Shortly afterwards Sullivan developed the technique of continuous intra-arterial infusion.[2] The idea behind the intra-arterial chemotherapy delivery was the 'first pass through effect' of the cytostatic agent. Morton and Eilber developed a combined modality for locally advanced limb sarcoma with three days regime of continuously intra-arterial doxorubicin with preoperative high dose fractionation radiation schedules of different dose levels in the late seventies.[3] Today we know however that intra-arterial chemotherapy has no advantage over systemic, intravenous, drug delivery.[4]

The theory behind the delivery of isolated regional chemotherapy is that a high drug uptake by the tumor may be achieved with higher doses of chemotherapeutic agents without increasing the systemic toxicity. The technique of isolated limb perfusion, a regional cancer treatment that allows the direct infusion of high doses of chemotherapeutic agents in the arterial supply of a tumor-bearing area of a limb, came available with the development of an extra-corporeal circuit for cardiac surgery. In the mid fifties the surgical-oncologists Creech and Krementz of the Tulane University in New Orleans developed the technique of the isolated limb perfusion.[5] In the sixties Cavaliere reported on selective heat sensitivity of tumor cells and added hyperthermia within the regional perfusion technique.[6] The ultimate goal of hyperthermia within the perfusion setting was increasing the blood flow and permeability of the cell membrane, therefore the drug uptake within the tumor by a synergistic effect, leading to an ultimate tumoricidal effect.

The three major advantages of the delivery of chemotherapy within the isolated limb perfusion setting are: 1) the so called 'first-pass' effect resulting in an increased drug uptake, 2) hyperthermia facilitating drug uptake by increased blood flow and permeability of the cell membrane and 3) the use of cytostatic agents which cannot be used outside the isolated limb perfusion setting due to the high systemic toxicity. Key point in isolated limb perfusion is that the dose of

26

chemotherapeutic agents used, can be 15-20 fold the maximum systemic tolerated dose, since vital organs are isolated from the perfusion circuit.[7] Since the first isolated limb perfusion was performed in the late fifties, the complex surgical technique is only used in a few cancer centers in the United States, Europe and Australia for the limb salvage treatment of locally advanced melanoma or soft tissue sarcoma.

This chapter will review the technical aspects of the isolated limb perfusion procedure, the currently available cytostatic agents, the assessment of tumor response, the clinical results of adjuvant and therapeutic isolated limb perfusions for various malignancies of the limb, as well as the local and regional treatment toxicity and future directions.

❑ Regional perfusion

Perfusion level

There are several levels for isolated limb perfusion in the upper and lower limb. Upper limb perfusions might be performed at the axillary level through the axillary artery and vein, or more distal upper limb perfusions through the brachial artery and vein. At the lower limb, perfusions might be performed at three perfusions levels, iliacal level through the external iliac artery and vein, femoral level through the femoral artery and vein, and at the distal thigh above the knee through the popliteal artery and vein. (Fig.1) The level of perfusion is determined by the involved part of the limb and the kind of disease, e.g. skin malignancies versus sarcomas. For skin malignancies the most proximal site of cannulation is the best choice, since the whole limb is at risk. In sarcoma the perfusion level is defined by a distinction part of the limbs containing the tumor. In case of limb recurrence(s) repeated perfusions might be possible, preferably at another level, to reduce the risk of complications to the previous exposed and perfused vessels.

Perfusion technique

During the perfusion the limb is exclusively perfused. After dissection of the appropriate artery and vein and ligation of the collateral vessels, to control

collateral flow and prevent leakage, the patient is heparinized systemically (heparin 3.3 mg/kg body weight).
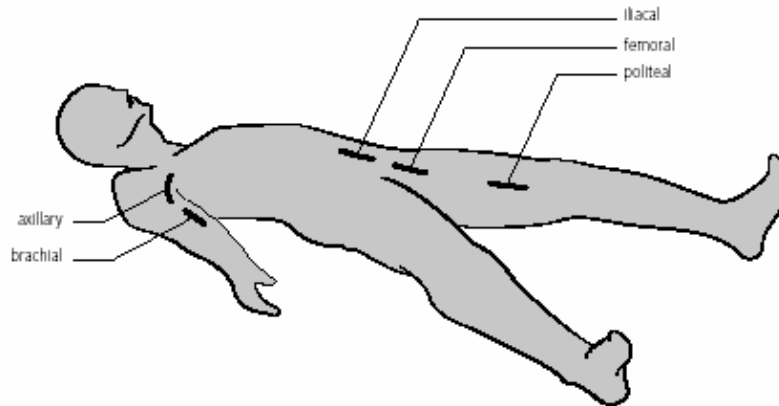
Fig.1 *Five different perfusion levels for regional perfusion of the extremities.*

The limb is isolated from the systemic circulation by an esmarch bandage twisted around the root of the limb and fixed around a pin inserted into the head of the humerus (axillary perfusion) or iliac crest (iliacal perfusion). An inflating tourniquet (300-400 mm Hg) is used for brachial or popliteal perfusions. The artery and vein are exposed, cannulated with 14 to 16 F catheters, connected to an extracorporeal circulation system and perfusion flow is initiated in the circuit. Thermister probes are placed in the subcutaneous tissue and muscle for continuous temperature monitoring. The perfused limb is wrapped in a thermal blanket to reduce heat loss. (Fig.2) The extracorporeal circulation perfusion system consists out of a roller pump, a membrane oxygenator, and a heat exchanger. The extracorporeal perfusion equipment improved during the last four decades with all

kinds of continuous data monitoring systems; recording of the temperature of the perfusate, the mean arterial and venous pressure in the perfusion canules, mean arterial pressure in the system, venous saturation and electronic balance of the perfusion volume. The perfusion group of the Groningen University Hospital further improved the perfusion technique by introducing the pressure-regulated perfusion, as well as safety by improvement in continuous leakage monitoring.[8,9]

The perfusate priming used in Groningen is oxygenated by a membrane oxygenator DIDECO 902 (DIDECO, Mirandola, Italy) with a gas mixture of air and O_2 , consisted of 250 ml Isodex in saline 0.9% (NPBI International BV, Emmer Compascuum, The Netherlands), 250 ml white cell-reduced (filtered) packed red cells, 30 ml of 8.4% $NaHCo_3$, 0.5 ml 5000 IU/ml heparin. Leakage into the systemic circulation is continuously monitored with radioactive tracers. A small calibration dose of radioactive Iodine-131 labeled human serum albumin, (RISA 0.5 MBq) and a dose of radioactive Technetium-99m labeled human serum albumin (RTcSA 10 MBq) are administered into the systemic circulation after surgical isolation of the limb is accomplished. The day before surgery the thyroid is saturated by the oral administration of iodine. A ten times higher dose of RISA (5MBq) is injected to the perfusion circuit. The 364-keV gamma rays emerging from the RISA and the 140-keV gamma rays from the RtcSA are measured with a NaI detector over the precordium. Leakage from the perfused limb to the systemic circulation results in an increase of the baseline level, continuously measuring the percentage of leakage from the perfusion solution into the systemic circulation. The risk of leakage is mainly related to the level of perfusion, and is in general less than 3%.[9]

The perfusions are flow regulated on the basis of arterial and venous pressure. To achieve adequate tissue perfusion during clinical regional perfusions, the extracorporeal circuit must be regulated at a delta pressure of 15 mm Hg between the pressure in the arterial and venous catheter.[8] Cytostatic agents are added directly into the arterial line of the perfusion circuit as soon as a limb temperature of 38^0 C is reached. Perfusions are performed during 60 minutes under mild hyperthermia (39^0-40^0 C). Adjustments of the flow rate and the pressure in the perfusion circuit by the perfusionist, as well as the blood pressure in the patient by the anesthesiologist, together with an optimal isolation of the limb by the surgeon, ensure a stable and therefore optimal perfusion.
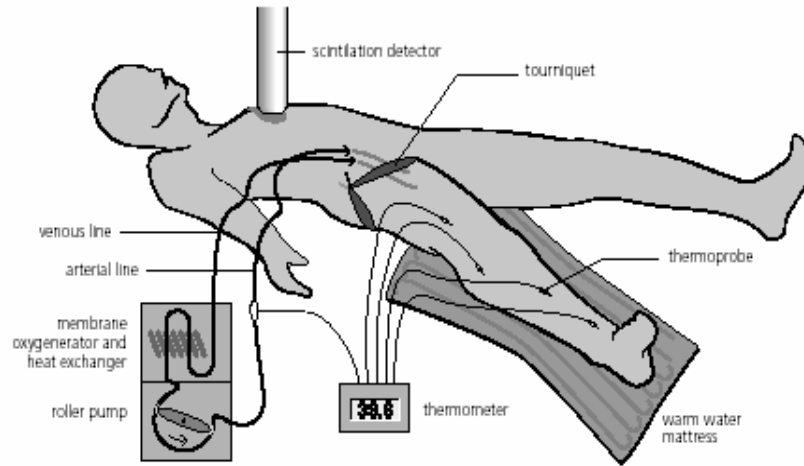
Fig.2 *Schematic drawing of a regional perfusion circuit for an iliacal perfusion of the lower extremity; esmarch bandage around the hip with Steinman pin inserted in the iliac crest, arterial and venous perfusion catheters connected to the arterial and venous line, membrane oxygenator, heat exchanger, roller pomp, leakage monitoring with scintillation detector placed over the heart, warm water mattress and thermo probes for skin and muscle temperature.*

When there is an increase leakage to the systemic circulation (losing), the flow rate should be reduced and the systemic blood pressure increased, eventually the tourniquet tightened, while with a loss of the systemic circulation into the perfusion circuit (gaining) the tourniquet should be tightened, flow rate increased and outflow 'occluded'.[9] In case of too much leakage, losing or gaining, the perfusion should be terminated for technical reasons to prevent eventual loco-regional or systemic complications. After the perfusion the limb is extensively washed out with 4-6 L of saline and the limb filled with 250 ml white cell-reduced (filtered) packed red cells. The vessels are restored, first the vein and secondly the artery to ensure adequate outflow of the limb. Finally the heparin is antagonized with prothrombin and a fasciotomy is performed. Patients perfused with tumor

necrosis factor α (TNFα) are monitored during 24 hrs on the intensive care, while patients perfused with melphalan are observed on the ward. No prophylactic antibiotics are prescribed. Patients receive subcutaneous low-dose molecular heparine till a full mobilization is achieved.

Cytostatic agents

Cytostatic agents used in isolated limb perfusion must have appropriate pharmacokinetic profiles such as steep dose-response curves without requiring metabolic activation. The most optimal cytostatic agents must therefore have a high degree of extraction within the perfused limb and a high total body clearance in case of systemic leakage. The real advantage of cytostatic agents used in the perfusion setting should be expressed by comparing the area under the curve of the concentration versus time profile using the perfusion drug application versus the conventional intravenous drug delivery.[10]

The first drug used in isolated limb perfusion by Creech and Krementz was melphalan.[5] A variety of other antineoplastic agents besides melphalan have been used during the last forty years in hyperthermic isolated limb perfusion such as darcarbazide (DTIC), actinomycin-D, thiotepa, mitomycine-C, doxorubicin and more recently cisplatin and carboplatin. The majority of the cytostatic agents were or ineffective, or the duration of response quite limited, or when a drug showed effectiveness the local toxicity hampered the further application. Doxorubicine showed effectiveness but disturbed the arterial vascularity of the limb, while cisplatin and carboplatin were too neurotoxic.[11-14]

Mephalan (L-phenylalanine mustard, GlaxoSmithKline, London, England) is an alkylating agent of the bischloroethylamine type compromising of nitrogen mustard and phenlylalanine. Phenylalanine is a metabolite of melanin and therefore melphalan specially targets melanocytes and melanoma cells. Its cytotoxicicity appears to be related to the extent of its interstrand cross-linking with DNA. Like other bifunctional alkylating agents, it is effective against both resting and rapidly dividing tumor cells. Melphalan is widely used since the sixties and the standard, most effective drug in the isolated limb perfusion setting for melanoma.

The dose calculation of chemotherapeutic agents in the past performed was on body weight. The dosage melphalan was 1.0–1.5 mg / kg for the lower limb and 0.5–0.7 mg / kg for the upper limb. The maximally tolerated dose of melphalan by this method of calculation without regional toxicity is thought to be 1.75 mg / kg, while 2.0 mg / kg will lead to severe regional toxicity.[15] Today the dosage is based on the perfused limb volume which seemed to be more appropriate.[16] The volume of the perfused limb is determined before surgery by immersion. The current melphalan dosage used is 10 mg / L limb volume for the lower limb and 13 mg / L limb volume for the upper limb. The maximal tolerable dose is 16 mg / L limb volume. Doses greater than 150 mg per limb results in dose-limiting regional toxicity.[17]

The systemic use of Tumor Necrosis Factor-alpha (TNFα; Boerhinger-Ingelheim GmbH, Vienna, Austria) is a pleiotrophic cytokine was abandoned due to its vasoplegia effect resulting in a severe septic shock syndrome. To use the potential effect of TNFα, e.g. tumor vessel destruction while limiting the side effect, e.g. septic syndrome. In the late nineties Lejeune introduced TNFα in the perfusion setting together with melphalan in the treatment of locally advanced melanoma and sarcoma of the limb.[18] The exact working mechanism of TNFα is still unknown, but progress is being made. TNFα attacks the neo-vascular endothelial cells in particular the tumor vasculature with rapid elimination of tumor blood flow during the treatment. Binding of TNFα to its endothelial receptors induces a cascade of mechanisms which suppress anticoagulant mechanisms and support thrombus formation in the tumor vessels, causing circulatory stasis and ischemia inside the tumor, followed by necrosis of the tumor. E-selectin, VCAM, ICAM-1, tissue factor and deactivation of an integrin ($\alpha_v\beta_3$) are all contributors in this cascade. Morphological changes of the endothelial cells with overlapping and elongation of the cell results in increased vessel permeability, facilitating the drug uptake of melphalan within the tumor cells.[19]

Is it possible to hence tumor response to TNFα? Endothelial monocyte-activating polypeptide II (EMAP-II) is a novel tumor-derived cytokine that sensitizes tumor vasculature to the effects of systemic TNFα. Tumor necrosis factor receptor I (p55) is upregulated on endothelial cells by exposure to the tumor-derived cytokine EMAP-II. Furthermore EMAP-II induces apoptosis and has antiangiogenetic effects. In an experimental rat-model Eggermont and co-workers showed indeed

32

the improved antitumor response to isolated limb perfusion with TNF*α* after upregulation of EMAP-II in STS.[20]

Since the introduction of TNF*α* in the perfusion setting various clinical and experimental studies have been performed to get more insight in the working mechanism of TNF*α*. Pre- and postperfusion angiography clearly showed that TNF*α* produces a selective destruction of the tumor-associated vessels, while the normal vasculature remained intact.[21] The dosage of TNF*α* is 3 mg for the upper limb and popliteal perfusion, while 4 mg is used for the lower limb. None randomized studies showed however that 1 mg TNF*α* might be even effective as the usual used 3 or 4 mg dosage.[22] Melphalan concentrations in the perfused limb decrease from time zero to 10-20% of the initial dosage by 60 minutes. In the initial first 5-10 minutes there is a rapid decrease of melphalan due to the uptake by the tissue, while during the remaining perfusion time there is a continuously degradation of the drug as well as an adherence to the plastic tubes surface of the perfusion circuit. To receive an optimal effect of the melphalan a minimal perfusion time of 45 minutes is required. In contrast TNF*α* is almost immediately binded to the pathological endothelial cells, and a so-called 'TNF priming time' of 15-30 minutes prior to the intra-arterial delivery of melphalan seems appropriate. Based on experimental studies the clinically used perfusion time for melphalan alone is 45-60 minutes and for the combined TNF-melphalan perfusion 60-90 minutes.[23]

Assessment of local tumor response

Clinical assessment of tumor response is defined by the World Health Organization criteria.[24] Complete response (CR) is defined as the disappearance of all measurable disease in the limb for longer than four weeks, partial response (PR) as regression of the tumor size by >50% for longer than four weeks, and no change (NC) as regression of <50% of the tumor or progression of <25% for longer than four weeks. Assessment is possible by physical examination and/or radiodiagnostic imaging. An MRI is used to measure the response in size for sarcoma. Today there are techniques to measure non-invasive in-vivo responses available. The currently most widely and available technique is Positron Emission

Tomography (PET) which measures the tumor metabolism.[25] New promising techniques such as bioluminescent imaging (BLI) are under development.[26]

The perfusion technique is a local regional cancer treatment with the ultimate goal to improve the limb salvage rate in locally advanced limb melanoma and sarcoma. There are no randomized studies performed as ultimate proof of the concept but the advantage of the technique in the limb salvage rate and local control of the disease is demonstrated in the various studies. The question if assessment of tumor response after isolated limb perfusion is a predictor of disease outcome is still unanswered. PET studies performed after TNF perfusions for locally advanced sarcomas provided insights in the effect of treatment, varying from CRs to NCs. Pre- and postperfusion Positron Emission Tomography (PET) showed as a result the rapid disappearance of tumor hypermetabolic areas linked to the hypervascularization.[27,28] Extensive pathological examinations of resected soft tissue sarcomas showed however in the majority of the specimens 'vital' tumor cells at the surrounding of the necrotic specimen.[27,28] Clinical, as well as in-vivo assessment of tumor response is a valuable predictor for the final effect of the regional treatment, which is a matter of course not translated to the overall outcome of the disease.

❑ **Isolated limb perfusion**

Melanoma

In the beginning isolated limb perfusion was used as a therapeutic treatment for locally advanced melanoma, recurrences and/or intransit metastases. Later on adjuvant limb perfusions were introduced in the treatment of melanoma of the limb. Over the last three decades many papers on hyperthermic isolated limb perfusion for melanoma combining adjuvant perfusions with therapeutic perfusions, often with different treatment schedules have been published and no conclusion could be drawn.[29] Finally a randomized trial showed (EORTC 18832/WHO-15/NAPG-1) no real benefit of hyperthermic isolated limb perfusion as an adjuvant treatment modality for patients with high-risk stage I melanoma (> 1.5 mm Breslow thickness).[30] On the otherhand isolated regional perfusion with melphalan is a well accepted treatment for intransit metastases and/or recurrences

in patients who are no longer candidates for local treatment, e.g. surgery, cryotherapy or laser treatment. Complete response rates of 40-60% with an overall response rate of 80% might be expected after therapeutic perfusions, while the addition of mild hyperthermia adds little to a normothermic perfusion.[31] Although double perfusions might increase the reponse rate further, this will have however no effect on frequency, time to local recurrence as well as time to distant failures, or survival.[32] Patients who did not achieve a complete response do worse than patients with a complete response. Roughly one third of the complete responders will recur, an additional one third will develop distant recurrences and the remaining one third will remain disease free. Perfusions performed in patients with recurrences after previous perfusions might render one third of these patients again disease free.

Other chemotherapeutic agents have been used in melanoma perfusions, showing lower subjective response rates and often higher toxicity. The most effective systemic agent in the treatment of melanoma is DTIC, but used in the perfusion setting this agent leads to complete response rate of 11% and a partial response rate of only 26%.[33] Cisplatin is one of the most successful alternatives with of 50-60% response rates, but showed a too high frequency of peripheral neuropathy.[13]

After the initial successful experience in the early nineties of isolated limb perfusion for melanoma with TNFα, gamma interferon, and melphalan (TIM) with 90% CRs, Lejeune and co-workers initiated a prospective randomized phase II study of patients with advanced melanoma of the limb by comparing TIM-perfusion versus TM-perfusion. The study showed a 10% drop in CR when interferon was omitted, but the difference was not significant.[34] A comparison with matched cases from a databank confirmed that melphalan only results in 52% complete responses.[34] The study was early terminated due to the fact that TNFα and melphalan showed overall no real benefit, compared to melphalan alone with a median survival of 2.5 years. Patients with low tumor burden or small tumors showed equivalent results were documented (TNFα and melphalan versus melphalan), while TNFα and melphalan showed higher response rates in bulky melanoma. Therefore melphalan is the drug to be used in melanoma perfusion and combined with TNFα when bulky disease or recurrent disease after previous melphalan perfusion is the indication for perfusion. The key question if a TNF

perfusion has advantage over a perfusion with melphalan alone is still unanswered, and results from the American College of Surgeons melanoma perfusion trial (03-C-0137) are pending.

Sarcoma

Soft tissue sarcomas (STS) are relatively rare malignancies of different mesenchymal derivation, accounting for less than 1% of all cancer in adults. Roughly fifty percent of all sarcomas are located in the extremities, and fifty percent of them are over the age of 65 years.[34] Although isolated limb perfusion was developed in the treatment of melanoma of the limb, the procedure was shortly after the introduction also applied in the treatment of soft tissue sarcoma of the limb. In the first experience Krementz showed an early response rate after melphalan of 83%, however complete regression of the tumor was hardly seen.[36]

The first breakthrough in the limb saving treatment of sarcomas was achieved in the eighties, when limb saving treatment, with or without adjuvant radiation treatment, showed the same disease free and overall survival as amputation.[37] The isolated limb perfusion program at the Groningen University, as elsewhere, was stopped, when the results achieved with the complex perfusion technique were not better than after surgery and adjuvant high dose irradiation.[38,39]

Although the combined modality treatment of surgery and adjuvant radiation improved the limb salvage rate of limb sarcomas, an amputation of the limb was still unavoidable in 5-10% of these patients. Other perfusions agents in the treatment of limb sarcomas were therefore investigated. Rossi claimed efficacy of doxorubicin in the perfusion setting for limb sarcomas, while another study proved that doxorubicin alone was ineffective and combined with melphalan too toxic. [40,41] Cisplatin showed also to be ineffective in the limb perfusion setting of sarcomas. [12,42] Although adjuvant chemotherapy may reduce the local failure rate in some patients, (neo) adjuvant and adjuvant chemotherapy had no beneficial effect in improving the limb salvage rate, disease free and/or overall survival in extremity sarcoma.[43]

The second breakthrough in the treatment of locally advanced soft tissue sarcoma of the limb came with the introduction of TNFα and melphalan in the perfusion setting by Lienard and Lejeune in the early nineties.[18] After TNFα perfusion, remarkable tumor shrinkage might be encountered within 6-12 weeks. Irresectable

sarcomas become resectable. Complete response rate of 18% and partial response rates of 64% by measuring the tumor size was achieved.[44] Various reports have shown that a limb salvage rate of roughly 80% can be achieved in patients with primarily irresectable limb sarcoma. Since the resection margins in these tumors are minimal, and often viable tumor cells are encountered at the periphery of the tumor adjuvant radiation is applied to ensure local tumor control. Adjuvant irradiation is well tolerated after previous intensive combined modality treatment of perfusion and extensive surgical resection.[45] TNF perfusion is now applied over a decade and in surviving patients we encounter now the long term treatment related morbidity necessitating amputation.[46]

A large European study proved the ILP concept in the limb salvage procedures for irresectable STS with TNFα and melphalan. The objective response rate was 76%. A limb salvage rate of 71% was achieved with a minimal treatment related morbidity.[47]An independent review committee considered that 80% of all enrolled patients in this study met indeed the criteria for irresectability and survival curves based on a match control study with cases of the Scandinavian Soft Tissue Sarcoma Databank showed that TNFα had no negative effect on survival.[48] Further analysis showed that ILP-patients survived as long as matched controlled conventionally treated patients.[49] The outcome of the ILP procedure in 'elderly' sarcoma patients was in general not different from the 'younger' sarcoma patients.[50] Perfusions in elderly limb sarcoma patients can on the otherhand sometimes not be performed due to atherosclerotic changes in the main arteries and/or severe co-morbidity. For these patients an amputation of the limb is unavoidable. With the improved surgical perfusion techniques, the perioperative care and intensive care facilities, perfusion treatment related morbidity for limb sarcoma is minimal.

Lymph edema-associated angiosarcoma, the Stewart-Treves syndrome, may be diagnosed in patients who underwent a mastectomy for breast cancer or axillary dissection for melanoma. The Stewart-Treves syndrome is extremely rare and the pathogenesis is not completely understood. The TNFα perfusion is an excellent indication for the limb salvage treatment for patients who where previously candidates for ablative surgical procedures.[51]

The results of the multicenter TNF study performed in the nineties lead to the approval of using Beromun® (Boerhinger-Ingelheim GmbH, Vienna, Austria) and

melphalan. (GlaxoSmithKline, London, England) for isolated limb perfusion treatment of locally advanced extremity sarcomas. Beromun® is not registered in the United States. Currently isolated limb perfusion with TNFα is worldwide available in more than 30 Centers. In 2002, 350 so called TNF-perfusions were performed. Why do not all the perfused sarcomas respond after a TNFα? Multidrug resistance is a major issue in chemotherapy treatment. Two groups investigated separately the expression of multidrug resistance in patients undergoing TNFα perfusion treatment. Hohenberger et al. investigated the expression of multidrug resistance genes major vault protein (MVP), MDR1, and MDR-associated protein 1 (MRP1) before, during and after isolated limb perfusion for sarcoma or melanoma. In 83% of the patients, MVP expression was induced during perfusion, while inductions of MDR1 and MRP1 were observed in only 13% and 27%. The temperature and the drugs were therefore unable to induce MDR1 and MPR1 in the majority of these tumors in the perfusion setting.[51] Komdeur et al. investigated the expression of P-glycoprotein (P-gp), MDR1, and lung resistance-related protein (LRP) in relation to the clinical outcome of TNFα perfusion treatment for extremity sarcomas. The sarcomas were more often positive for P-gp, than for MRP1. The MDR status was not predictive for tumor response after TNF-ILP. Data of the study showed also that TNFα perfusion did not induce MDR positive sarcomas.[52] This is an important finding, since systemic doxorubicin based polychemotherapy is currently investigated in soft tissue sarcoma patients after TNFα perfusion as an adjunct within the EORTC trial 62931. The combination of TNFα and melphalan is currently the standard drug combination in isolated limb perfusion for the limb salvage treatment of primarily irresectable soft tissue sarcoma of the extremities, but there are still 20-30% of the tumors that will not respond. Therefore agents synergistic with TNFα must be investigated such as doxorubicin, or actinomycin-D. The combination of TNFα and doxorubicin was recently clinically investigated showing no benefit when compared to TNFα and melphalan.[54] Seynhave et al. showed in an experimental perfusion study in the rat-model that the combination of actinomycin-D and TNFα improved the tumor response in the soft tissue sarcoma bearing rats. The responses were unfortunately accompanied by severe, dose limiting, local toxicity; a synergistic anti-tumor response with idiosyncratic locoregional toxicity to the normal tissues.[55] These

experiments don't warrant the further clinical investigation of these two drugs in isolated limb perfusion for sarcomas.

Miscellaneous tumors

Isolated regional perfusions with TNFα have been successfully performed for in patients with locally advanced squamous cell carcinoma and Merkel carcinoma not amenable to local surgery with response rates of 87% (CR 60%, PR 27%).[56] There is also a limited perfusion experience with cisplatin, as well as with TNFα for unresectable bony sarcomas.[12,57] No conclusion can be drawn from these studies.

❑ **Treatment toxicity**

The treatment toxicity can be categorized as side effects from systemic exposure to the cytostatic agent and as side effects due to the regional effects of the high-dose exposure and hyperthermia. The vast majority of perfusions can be performed with systemic drug exposure of less than 3%.[9] The systemic exposure depends not only on the adequacy of the isolation of the limb during the perfusion, but is also related to the systemic exposure to the perfused agent during reperfusion.[58] Although the limb is flushed after the perfusion, residual active agents still remain in the limb within the intravascular space or in the interstitial fluid, which results in a systemic peak of drug concentration following the re-establishment of normal vascular flow to the extremity. Systemic leakage of melphalan might cause mild nausea, bone marrow depletion, and fever. In case of leakage over 10% patients should have their white blood counts measured 7 to 14 days post-operatively to monitor bone marrow depression.

TNFα might induce secondary host mediators in contrast to the other drugs used in the perfusion setting.[59] The most serious complication after TNFα perfusion is the systemic inflammatory response syndrome (SIRS) accompanied by fever, rise in cardiac output, fall in systemic vascular resistance and the need for fluid resuscitation and inotropes. If leakage exceeded the 2% limit during perfusion, there was less exposure of the tumor bearing limb to TNF and an increased exposure of the patient systemic circulation to TNFα, resulting in more systemic side effects.[58] There is a direct correlation between maximum TNFα concentrations

and systemic vascular resistance and cardiac index.[60,61] Severe toxicity might be encountered over a leakage of 5%.[18] In contrast Stam et al observed only a mild postoperative toxicity in the event of a significant leakage during perfusion.[62] The more experience is achieved with the so called TNFα -perfusions, the better the transient, systemic side effects could be managed during the perfusion and postoperatively with appropriate resuscitative techniques. The leakage should not extend 10% (as per licensee). Currently SIRS is only seldom seen, since all the institutions performing TNFα perfusions are experienced, achieve less leakage rates and use extensive washout procedure at the end of the perfusion procedure, to reduce the release of TNFα from the perfused limb to the main circulation after restoration of the limb circulation.

The normal tissues in the limb, skin, subcutaneous tissue, muscle, nerves, blood vessels, bone, and cartilage are all exposed to the same concentrations of cytostatic agents active against the tumor. Wieberdink developed a regional toxicity scoring system.[15] The effects of the perfusate on normal tissues varied widely between individuals. Melphalan may cause skin toxicity, erythema and blistering, and serves as a document of the distribution of the perfusion.[63] When a lymph node dissection is performed together with the perfusion, edema of the involved limb might be encountered. The most important toxicity is related to muscle and nerve damage, which might be avoided by a prophylactic fasciotomy, preventing the development of a compartment syndrome.[64] All patients undergo some degree of skin reaction and lymph edema TNFα after the perfusion. This resolves in general within a month regarding the accompanied procedures, e.g. tumor resection and/or radiation. The Rotterdam perfusion group documented functional impairment of 20% of the upper limb and 36% of the lower limb after melphalan perfusion.[64] In contrast Olieman and co workers showed no impairment of limb function after melphalan perfusion for melanoma compared to the unperfused limb.[65] It might be expected that small proportion of patients (5-8%) undergoing a limb perfusion for melanoma will have long-term limb symptoms, without severe impairment, secondary to their perfusion treatment.[66] After perfusion for locally advanced sarcoma impairment of limb function is not related to the perfusion, but to the extend of the surgical resection of the soft tissue mass and eventually adjuvant irradiation. Another risk factor in isolated regional perfusion is not related to the cytostatic agents, but to the (elderly) patients' vascular status and the

40

extent of the soft tissue mass in case of perfusion for a locally advanced soft tissue sarcoma. Manipulation, cannulation and tight occlusion of sclerotic vessels might cause embolic events, arterial stricture after vessel repair, or arterial thrombosis, requiring reoperation or even amputation of the perfused limb. Beside the arterial complications, from the venous side deep venous thrombosis is sometimes encountered due to cannulation of the vein or the thrombogenic side effect induced by melphalan. Increase in limb temperature might slightly increase limb toxicity in melphalan perfusions. The addition of TNFα will significantly increase the limb toxicity due to increase of melphalan into the tissues and not to microembolization of tumor cells.[67,68]

❑ Summary

Isolated limb perfusion has the potential to the deliver high doses of chemotherapeutic agents to a tumor-bearing limb. There is no evidence-based indication for adjuvant isolated limb perfusions for high-risk limb melanoma, in contrast to therapeutic perfusions for extensive local recurrences or intransit metastases. The introduction of TNFα in combination of melphalan redefined the indication for therapeutic and palliative isolated limb perfusions in the limb saving treatment of locally melanoma and sarcoma of the limb, as well as multifocal skin cancers and drug-resistance bony sarcomas. The currently most widely used drugs in the perfusion setting are melphalan with or without TNFα.

Various aspects of isolated limb perfusion treatment need to be further explored. The future developments in regional perfusion research should focus on new therapeutic perfusion agents and/or increasing the tumor sensitivity for TNFα without decreasing tumor response, as well as the further exploration of new anatomical areas in the body for regional cancer treatment, such the liver, lung, kidney and pelvis.

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Chapter III

❑ Hyperthermic isolated regional perfusion of the limb with carboplatin

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❑ Introduction

Hyperthermic isolated regional perfusion of the limb (ILP) is a technique which has the possibility to deliver high concentrations of chemotherapeutic agents to an extremity, whereas only low drug concentrates are reached in the systemic circulation. Since Luck introduced melphalan (L-phenylalanine mustard) in 1956 as an active agent to inhibit growth of malignant melanoma in mice, it has been the preferred agent in ILP for intransit metastases of melanoma.[1] Although ILP with melphalan is effective in the treatment of the patients with intransit metastases of melanoma, with a complete response rate of 50%, it has no effect in the adjuvant setting.[2-4] Newer drugs which might be more effective than melphalan in the treatment of melanoma by ILP have therefore been sought. Cisplatin is one of the most active drugs in cancer treatment, however it has serious dose-limiting toxicity such as neuro-, nephro-, and ototoxicity.[5] Therefore ILP with cisplatin was evaluated as an alternative to overcome these limitations of systemic treatment in the treatment of recurrent melanoma of the limb. However the effectiveness of cisplatin in the so called therapeutic perfusion setting and for local control of tumor growth for the treatment of locally advanced melanoma and sarcoma of the extremities with delayed excision is not impressive.[6-10] And the local neurological side effects of ILP with cisplatin in the perfused limb are still present in the form of a irreversible peripheral sensory and motor neuropathy.[7] Therefore in several institutions cisplatin was abandoned as a cytostatic agent in the perfusion setting. There are however several other institutions that still use and advocate cisplatin in ILP.[11-13] Carboplatin is a second generation platinum analogue with similar efficacy in most tumors but with less neuro-, oto- and nephrotoxicity when compared to cisplatin when applied systemically.[14,15] A feasibility study was initiated to study the effect of carboplatin, as an agent for ILP with respect to the local tumoricidal effect, as well as the local treatment morbidity.

❑ Patients and Methods

Between November 1991 and February 1993 two patients (patient 1 and patient 2) with locally advanced recurrent and intransit metastases of melanoma of the lower

limb (Stage IIIA, MD Anderson staging) and one patient (patient 3) with a locally advanced primary irresectable soft tissue sarcoma of the lower limb participated in a feasibility study of ILP with carboplatin. None of the patients had distant metastases at time of the perfusion. Patients were treated with intent of preserving the affected extremity. The maximum follow up period was 95 months. Patient data are summarized in Table 1. Since the systemic dosage of carboplatin used clinically is 4 times the cisplatin dosage, a fourfold higher dose compared to cisplatin was used for the limb perfusion.[5,16] Based upon an earlier dose finding study where a maximum dose of cisplatin of 30 mg/L limb volume was used in ILP, an initial carboplatin dosage was chosen of 125 mg/L limb volume.[7,9]

Patient No.	Age (yrs)/ Sex	Tumor type and staging*	Previous therapy	Perfusion level	Limb vol (l)	Total carboplatin in mg
1	57/F	melanoma stage IIIA	tumor excision + ILP with melphalan + actinomycin-D	popliteal	5.1	625 mg
2	72/M	melanoma stage IIIA	tumor excision	femoral	12	1500 mg
3	52/M	low grade myxoid liposarcoma	None	iliacal	13	1625 mg

M = male, F = female; *Staging according to M.D. Anderson staging system

Table 1: Patient characteristics, perfusion characteristics and carboplatin dosage

All patients had normal pre-operative kidney function. To prevent postoperative renal failure due to systemic leakage, hyperhydration was started one day before ILP and was continued up to 5 days postoperatively. Hyperhydration consisted out of 2 l 0.9% NaCl intravenously superfluous to the patients' habitual fluid intake. The perfusion technique used at the Groningen University Hospital is based on the technique developed by Creech et al.[17] All patients were operated under general anaesthesia. After a skin incision, the iliac, femoral or popliteal vessels were exposed and collateral vessels were clipped. The patients were heparinized (3.3mg/kg body weight) and catheters were inserted in the artery and vein, and both were connected to an extracorporeal circuit. The extremity was completely isolated from the central circulation with the aid of an Esmarch bandage. The perfusate consisted out of 250 ml red blood cell concentrate, 250 ml Isodex® (NPBI, Emmer-Compascuum, The Netherlands) in 0.9% NaCl, 30 ml NaHCO$_3$ 8.4% and 25 mg heparine (2500 IU) (Braun Melsunger AG, Melsunger, Germany). Leakage to the systemic circulation was assessed by injecting I[131]-albumin into the isolated circulation and continuously monitoring of radioactivity was performed by means of a NAC-scintillation detector, which was placed over the heart.[18] The leakage was indicated by extent of cross circulation between the perfused extremity and the circulation in the rest of the body. All perfusions were performed under mild hyperthermic conditions (39 - 40°C). If the muscle temperature had reached a temperature of 38°C and after leakage to the systemic circulation was excluded, the carboplatin 125 mg/L limb volume (Bristol Myers Squibb Company, Woerden, The Netherlands) was administered to the perfusate over a 10 min period. The flow rate of the perfusion fluid in the perfusion circuit was approximately 500 ml/min. After 1 hour of perfusion, the extremity was flushed with 500 ml Isodex® in 0.9% NaCl and 250 ml red blood cell concentrate. Both catheters were removed and the vessels were repaired. The heparin was then neutralized with protamine sulfate and a fasciotomy was performed to prevent a compartment syndrome. Before and during the perfusion, 10 ml perfusate samples were collected at 10 min intervals to determine the ultrafiltrated platinum (fPt) levels as previously extensively published.[19] The leakage into the systemic circulation was also determined by measuring the patients fPt plasma concentrations at the end of each perfusion procedure both before and after restoration of the circulation to the perfused limb and during the 7 days following

54

the perfusion. For fPt perfusate elimination kinetics, data were subjected to logarithmic regression analysis (concentration = $A.e^{-k.t}$). The areas under the concentration *vs.* time curves (AUC) were calculated using the model independent trapezoidal rule and covers the perfusion period (t = 0 - 60 min).[20] Tissue biopsies were taken for fPt determination in both melanoma patients at the end of the ILP, when the normal limb circulation was restored.

All three patients were followed up post operatively by history, physical examination, and routine blood counts and blood chemistry analysis were performed on a daily basis on days 1 - 7, and 14 and 21 days postoperatively. Total serum protein and albumin was controlled one day preoperatively and postoperatively. Six weeks after perfusion electrodiagnostic evaluations (electromyogram (EMG) and nerve conduction study) were performed to investigate nerve toxicity of the carboplatin perfusion in patients 1 and 3. Unfortunately patient 2 refused the neurological examination. The local limb perfusion toxicity was graded according to the criteria decribed by Wieberdink et al.[21] Eight weeks after perfusion, patient 3 was readmitted for a so called delayed resection of a locally advanced soft tissue sarcoma of the lower limb. The study was approved by the local Medical Ethical Committee of the Groningen University Hospital and all patients gave informed consent.

❑   **Results**

All three perfusions could be performed under standard perfusion conditions with minimal I^{131}-albumin leakage (0-5%) (Table 2) and without any acute systemic toxicity or morbidity. The local toxicity consisted in all 3 patients of a grade II toxicity of the perfused limb which is characterized by erythema, edema or loss of sensation which resolved spontaneously in all patients.[21] Patient 2 developed an abscess in the groin area of the perfused limb 3 weeks after the femoral perfusion with superficial inguinal node dissection. Except for a marked decrease in total serum protein and albumin 1 day postoperatively, there were no major biochemical changes or myelosuppression. The total serum protein decreased from a mean of 67.3 $\pm$ 2.5 g/L preoperatively to 47.7 $\pm$ 4.5 g/L (P<0.04 paired Student's *t*-test) one day postoperatively. Serum albumin decreased from a mean of 46.3 $\pm$ 4.2

Patient No.	Leakage (%)	Toxicity grade*	Clinical signs of neuropathy	Electrodiagnostic evaluation (EMG + nerve conduction)
1	1.8	II	yes	Sensory + Motor neuropathy
2	0	II	yes	nd
3	5	II	yes	Sensory + Motor neuropathy

*nd = not determined; *Toxicity grade according to Wieberdink et al.[21]*

Table 2: *Leakage, toxicity and neurological follow up*

g/L preoperatively to 32.0 $\pm$ 4.0 g/L (P<0.002 paired Student's *t*-test) one day postoperatively. This resulted in the development of edema in the perfused limb postoperatively for which intravenous albumin was administered postoperatively in all three patients to correct the serum albumin. Physical examination of the first two patients, both melanoma intransit metastases, revealed clinically a complete response making delayed excision unnecessary. A CT scan of the liposarcoma of patient 3 showed a 25% reduction of the tumor size which allowed a complete macroscopical marginal resection after 8 weeks. However microscopically vital tumor was observed in at least 50% of the resected material with focal necrosis. The patient received adjuvant radiation therapy (60 Gy) at the primary tumor site, which was well tolerated. Clinically all 3 patients showed signs of sensory and motor neuropathy in the perfused limb. Electrodiagnostic evaluations were performed in order to assess the local neurotoxicity of the carboplatin in patients 1 and 3. Patient 1 was tested both pre- and postoperatively because this patient underwent an ILP with melphalan of the same limb one year previously. Preoperative nerve conduction velocities of the perfused left leg (peroneal nerve, F

56

response, H reflex) were all slightly slower then on the right side. The sensory sural nerve amplitude was also lower on the left side (left 4μV, right 16μV). The control EMG and nerve conduction study postoperatively showed a further decrease in the conduction velocity of the peroneal nerve and the amplitude from the sural nerve decreased by 2μV. The postoperative EMG and nerve conduction study of patient 3 revealed that the right (perfused limb) extensor hallucis muscle and abductor hallucis muscle showed partial denervation, and the sural nerve also showed a lower amplitude compared with the left leg.

In all three patients the affected limb could be preserved. After a complete initial clinical response to the perfusion, patient 1 developed local recurrences over the next few years and the first being within 1 year postperfusion. All of these local recurrences and intransit metastases were treated by local excision only. This patient is still alive 95+ months after perfusion with carboplatin. Patient 2 also showed a complete initial clinical response but within 1.5 year a local recurrence appeared at the primary tumor site which was excised. This patient went on to develop lung metastases 44 months after perfusion and died 56 months after perfusion. Patient 3 did not develop a local recurrence after radiation therapy but died from lung metastases 31 months after perfusion. Systemic plasma concentrations of fPt, determined at the end of perfusion before restoration of the normal circulation, were also found to be relatively low (<0.1 - 0.98 mg/L). Systemic plasma fPt concentrations remained negligible in all patients during the 7 days following the perfusion. Perfusion data and pharmacokinetic parameters are summarized in Table 1 and Table 3. Concentrations of fPt in tissue biopsies, taken from patients 1 and 2 of the skin and the anterior tibial muscle at the anterolateral part of the lower extremity during the fasciotomy after ILP, showed a great variation in concentration between the skin and muscle, with especially high fPt concentrations in the skin. In patients 1 and 2 the concentrations in the skin were respectively 27.6 ug fPt g^{-1} tissue and 25.0 ug fPt g^{-1} tissue compared to the concentrations in the muscle which were respectively 9.0 ug fPt g^{-1} tissue and 7.6 ug fPt g^{-1} tissue.

Patient	t½ (min)	Cmax (mg/L)	$C_{t=60}$ (mg/L)	AUC ((mg/L)/min)	systemic fPt concentration (mg/L)
1	104	488	344	24070	<0.1
2	30	824	113	9995	0.98
3	50	620	250	20378	<0.1

Table 3: *Pharmacokinetic parameters of fPt in the perfusate during 60 min ILP with 125 mg/L carboplatin extremity (assuming first order kinetics) and systemic fPt concentration after 60 min ILP.*

❑ Discussion

Regional isolation perfusion was introduced to deliver large doses of cytotoxic drugs to the local tumor area while avoiding systemic toxicity. Melphalan is still the most frequently used antitumor drug in ILP for melanoma.[6,8,22,23] Even with a high remission rate (80%), a great deal (50%) of the melanoma patients fail to achieve a durable complete response.[2,3,23] Therefore a search was initiated for alternative drugs or the application of combination of agents to be used in ILP. Several cytostatic agents have been used besides melphalan, such as actinomycin D and DTIC (imidazole carboxamide). The feasibility of cisplatin in ILP has also previously been investigated by several institutions, but with conflicting results. Some authors claim that the local severe toxicity and the equal or less effectiveness does not justify the use of cisplatin.[6-10] But there are also other institutions that still use and advocate the use of cisplatin in ILP.[11-13] Carboplatin, a second generation platinum compound, was attractive for clinical use because of its reduced nephro-, neuro-, and ototoxicity than cisplatin when administered systemically.[24] It differs in the molecular structure from cisplatin by replacing the two *cis* chloride atoms by

a cyclobutane dicarboxylate molecule.[5] Platinum co-ordination complexes inhibit tumor growth by their effects on DNA replication. Once bifunctionally bound to DNA, the mode of action of carboplatin is not different from cisplatin, but the platinum induced DNA lesions (DNA interstrand cross links) are formed at a slower rate with carboplatin compared to cisplatin.[16] Our clinical results show that application of carboplatin in ILP led to the development of, or an increase in the neuropathy in the perfused limbs of all three patients. In two patients this was confirmed by electrodiagnostic evaluation. Patient 1 already showed clinical signs of neuropathy in the affected limb which was confirmed by EMG and nerve conduction study prior to the carboplatin perfusion. This neuropathy was probably due to the previous perfusion with melphalan. The postoperative tests after the carboplatin perfusion of this patient showed no apparent denervation potentials, but increased disturbances in the motor and sensory conduction velocities compared to the first neurological tests were observed. Patient 2 and 3 had no clinical signs of a neuropathy preoperatively. However, the postoperative electrodiagnostic findings of patient 3 did show a new development of a neuropathy distally in the perfused limb. With the limitations of the small number of patients treated in this feasibility study it should be noted that with respect to the locoregional tumor control, the treatment regimen did not appear to be promising. Both melanoma patients who underwent perfusion with carboplatin developed local recurrency and/or intransit metastases within 1 year after perfusion, and histologically 50% vital tumor was still observed after excision of the sarcoma from patient 3.

There has only been one case report and one study in which carboplatin has been used for isolated limb perfusion. Nakayama et al. reported minimal side effects after perfusing 1 patient with 450 mg carboplatin for melanoma of the lower limb.[25] They found a complete clinical and histologic response of the tumor and in their follow up period of 10 months, no local recurrency was observed. This is in concordance with the findings of Ariyan et al. who performed ILP on 20 patients with carboplatin (400 mg/m^{2} body surface area) and the results were found to be effective and with very low toxicity with a median follow up of 25 months.[11] Both studies however do not report on observed local neurotoxicity. In our study the carboplatin dosage was calculated on the limb volume and not on the body surface area, thus leading to a much larger carboplatin dosis administered (up to 4 times

as much). This could possibly explain the enhanced neurotoxicity seen in this series.

Next to the search for new agents for ILP, different combinations of drugs are being investigated. The combination of tumor necrosis factor α (TNFα) and melphalan has been used for the local perfusion of recurrent melanoma of the limbs and for extremity sarcomas.[26-31] Lienard et al. reported that the complete response rate for melanoma metastases increased to 80 - 90% after the addition of TNFα to the perfusate, but with an increased risk of side effects.[31] The median disease free interval time did not increase with the addition of TNFα, making monotherapy with melphalan currently the drug of choice for ILP in melanoma.[23]

For soft tissue sarcomas of the limbs the combination of TNFα and melphalan as perfusion agents has been proven to be effective in salvaging the affected limbs.[27,28,30] Results from a recent international multicenter study, showed that in 246 patients with irresectable soft tissue sarcoma a complete response rate of 28%, a partial response rate of 47% and 17% no change after perfusion with TNFα and melphalan was observed. Enabling delayed excision and preserving the extremity in a substantial number (71%) of perfused patients.[32]

A combination of TNFα and cisplatin has been investigated by Bartlett et al. in peritoneal perfusion in the treatment of peritoneal carcinomatosis.[33] The results were disappointing because of an increased nephrotoxicity compared to cisplatin alone, rendering it unfeasible. A canine experimental study by van Ginkel et al. also combined the drugs cisplatin and TNFα in ILP and tested this regimen 6 healthy dogs.[34] They observed an increased toxicity compared to ILP with cisplatin alone. Despite a sublethal systemic concentration of TNFα that leaked from the perfusion circuit, 3 dogs died within 24 hours of ILP. Another dog developed a necrotic limb which the authors explained as an enhanced cytotoxic effect of cisplatin to the local tissues due to the addition of TNFα. Taking into account the before mentioned results between cisplatin and TNFα and considering the similarity between cisplatin and carboplatin, we do not warrant further investigation into the feasibility of the combination between carboplatin and TNFα.

The local neurotoxicity observed with ILP makes carboplatin an unattractive alternative agent for ILP. However, compared to the before mentioned studies of

Nakayama[25] and Ariyan[11] the patients in this study received a higher dose of carboplatin which could account for the observed neurotoxicity.

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62

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Chapter IV

❑   Continuous leakage measurement during hyperthermic isolated limb perfusion

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❑   **Introduction**

With hyperthermic isolated limb perfusion (ILP), high doses of cytotoxic agents can be administered locally in a limb without systemic toxicity. This method was introduced in 1958 by Creech and Krementz and has since undergone many improvements such as the addition of hyperthermia.[1,2] ILP is employed mostly in the treatment of recurrent melanomas and locally advanced extremity soft tissue sarcomas (STS). Since Luck introduced the cytostatic agent L-phenylalanine (melphalan) in 1956, it has been the preferred agent in ILP for intransit metastases of melanoma.[3] Because the dosage of the chemotherapeutic agent is high (up to 15–20 times the systemic tolerated dose), a considerable risk of toxic effects due to leakage of the perfusate to the systemic circulation may occur.[4] Leakage of more than 15% perfusate containing melphalan into the systemic circulation may cause toxic effects such as bone marrow depression, gastro-intestinal toxicity, hair loss and pruritis.[5-7] With the introduction of recombinant tumor necrosis factor α (TNFα) in the early nineties, the effectiveness of the ILP was increased for melanoma and especially for STS patients.[8,9] However, leakage of TNFα into the systemic circulation gives rise to more severe side effects than melphalan alone. A 1% leakage may already result in hypotension of the patient and a 10% leakage of TNFα can cause a potentially fatal septic shock like syndrome.[10,11] However, strict isolation of the limb is not always achievable due to anatomical variations and/or technical reasons. Minimal systemic leakage can therefore not always be prevented, and makes continuous leakage monitoring during the perfusion procedure of utmost importance. Recently many methods of leakage detection methods have been described in the literature ranging from intermittent blood sampling to continuous external monitoring using radio-isotopes.

At the Groningen University Hospital continuous external leakage measurement is performed using ^{131}I-albumin as the radio-isotope. Between 1977 and 1991 the median of the maximum percentage leakage was reported to be 8.0% in this institution.[7] Since that period there have been a number of improvements in the ILP technique and in leakage monitoring. We therefore embarked on a retrospective study to analyze the maximum leakage of the patients who underwent ILP in the period between 1991 and 2000.

68

❑ **Patients and Methods**

Between January 1991 and March 2000, 119 patients underwent a limb perfusion at the University Hospital Groningen. Of the 119 perfusions, 67 were iliacal (56%), 12 femoral (10%), 25 popliteal (21%) and 15 axillary (13%) perfusions. The perfusion was carried out on 71 female (60 %) and 48 male patients (40%) with a median age of 56 (range 17-79) years (Fig. 1). Sixty-two patients (52%) were perfused for intransit melanomas, 51 patients (43%) for a primarily unresectable sarcoma and 6 patients (5%) had other types of malignancies (Fig. 2). Patient, tumor and treatment data was retrieved from the Groningen melanoma and sarcoma databases.

Perfusion Technique

The perfusion technique used at the Groningen University Hospital is based on the technique developed by Creech *et al* and has extensively been described before by Schraffordt Koops *et al*.[1,12] The surgical technique of the ILP between the two time periods remained the same. All patients were operated under general anesthesia. After a skin incision, the iliac, femoral, popliteal or axillary vessels were exposed and collateral vessels were clipped. In iliacal perfusions the hypogastric vessels were also temporarily closed. The patients were heparinized (3.3mg/kg body weight) and catheters were inserted in the artery and vein, and both were connected to an extracorporeal circuit. The extremity was isolated from the central circulation with the aid of an Esmarch bandage. The perfusate consisted of 250 ml red blood cell concentrate, 250 ml Isodex® (NPBI, Emmer-Compascuum, The Netherlands) in 0.9% NaCl, 30 ml $NaHCO_3$ 8.4% and 25 mg heparin (2500 IU) (B.Braun Melsungen AG, Melsungen, Germany). All perfusions were performed under mild hyperthermic conditions (39 - 40°C). If the muscle temperature had reached a temperature of 38°C and if almost no leakage to the systemic circulation was detected, the chemotherapeutic agents were directly added to the perfusion circuit via the arterial line. Especially for the TNFα perfusions an almost 0% leakage was required before the TNFα was added to the perfusion circuit. For the less toxic melphalan, a leakage of 5% was generally accepted. mg/l upper limb volume (Alkeran®, Glaxo Wellcome, London, Great Britain). If TNFα was used (Boehringer Ingelheim International GMbH, Ingelheim am Rhein, Germany), the
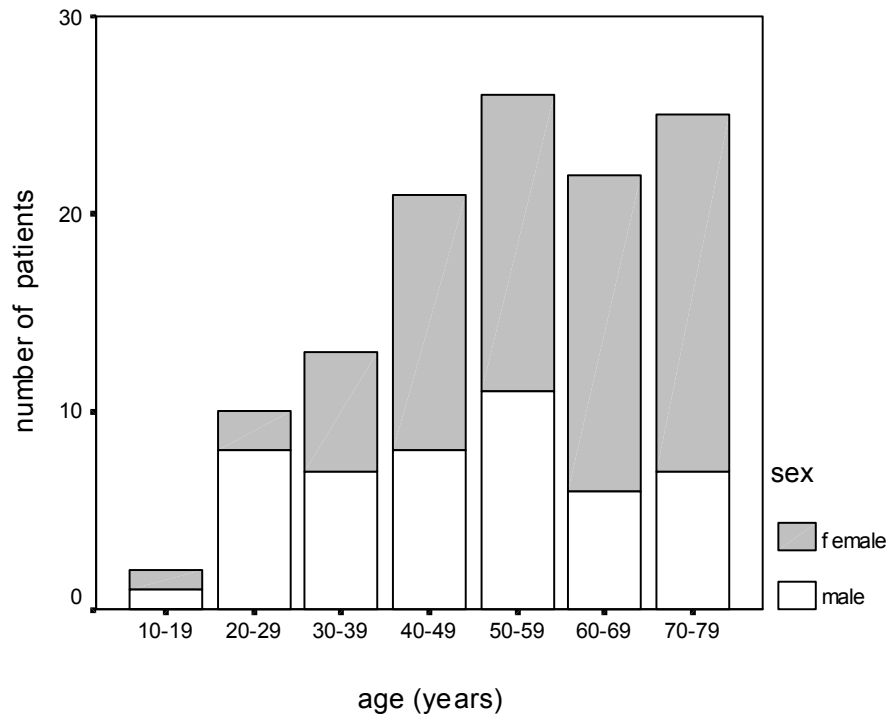
Fig. 1 *Age distribution of all 119 perfused patients*

dosage was 4 mg for the lower extremity and 3mg for the upper extremity (given 30 min before the melphalan).The dosage used in melphalan perfusions is 10 mg/l lower limb volume and 13 The flow rate of the perfusion fluid in the perfusion circuit was approximately 500 ml/min. After 1 hour for non TNFα perfusions and 90 min for TNFα plus melphalan perfusions, the extremity was flushed with Isodex® in 0.9% NaCl (between 3-6L depending on the level of perfusion) and 250 ml red blood cell concentrate. Both catheters were removed and the vessels were repaired. The heparin was then neutralized using protamine sulfate and a fasciotomy was performed to prevent a compartment syndrome. If required, a local excision of the melanoma and intransit metastases was performed after the perfusion and, if necessary a free skin graft from the other limb was taken and kept under sterile conditions at 4 degrees Celsius and placed on the wound 3 days later. Post ILP patients were admitted to the intensive care unit for 24 hours in order to monitor clinical toxicity from the TNFα and/or melphalan.

70
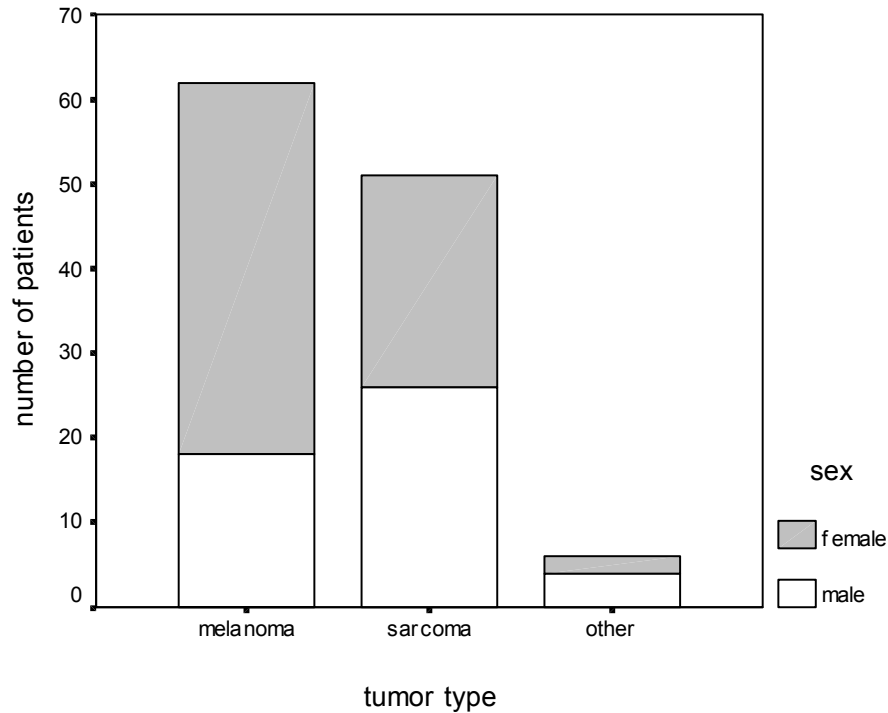
Fig. 2 *Tumor types corresponding to gender*

Leakage Monitoring

Leakage measurement took place with the help of radioactive tracers. A low dose of ^{131}I-albumin (0.5 MBq) and a dose of ^{99m}Tc-albumin (10 MBq) were administered to the systemic circulation. A tenfold higher dose of ^{131}I-albumin (5 MBq) was administered to the isolated limb circulation. A fixed scintillation detector placed above the heart measured the radioactivity over the cardiac zone. The count rate of ^{131}I-albumin at the start of the perfusion (t=0) was the setpoint used for calculating the leakage percentage of the cytotoxic drugs. Leakage from the perfused limb to the systemic circulation results in an increase of this count rate. This increase, corrected for the blood volume ratio and the radioactivity ratio in both compartments, was a direct measure of percentage leakage. These changes were automatically converted by a microprocessor connected to the detector into percentages of leakage. Radioactivity was continuously registered during the

71

procedure (fig. 3). The ^{99m}Tc-albumin baseline radioactivity was used as a control for the detector efficacy during the whole procedure. To avoid detrimental effects by the radioactive ^{131}I to the thyroid gland, the patient was given iodine (15 drops of Lugol solution twice daily) 1 day before the operation.

Statistics

The software package SPSS 9.0 for Windows (SPSS, Inc., Chicago IL) was used for statistical analysis. The maximum leakage is defined as the zenith of the leakage that occurred during perfusion in each patient. For descriptive statistics the median value of the maximal leakage was documented. The Mann Whitney U test was used for comparison of the differences in maximum leakage between the various subgroups.

❑ Results

All perfusions were performed under standard perfusion conditions. There was no mortality related to the perfusions. For all 119 patients the median value of the maximum leakage (leakage$_{max}$) was 2.7% (range 0-21.0%). There was no statistical difference in leakage$_{max}$ between male (2.4%, range 0-21.0%) and female (3.0%, range 0-12.0%) patients. Nor was there a statistical significant difference in leakage$_{max}$ between the various age groups. However, a statistical difference of the leakage was detected between the levels of perfusion. Iliacal and femoral perfusions had significant higher leakage$_{max}$ than popliteal and axillary perfusions. For iliacal perfusions the leakage$_{max}$ was 4.5% (0-21.0%), for femoral perfusions 3.8% (0-15.5%), for popliteal perfusions 1% (0-9.0%) and for axillary perfusions 0% (0-4.0%) (Fig. 4). The leakage$_{max}$ for the groups stratified for the different drugs used also showed a statistical significant difference. When TNFα was used, a lower leakage$_{max}$ was detected compared to when no TNFα was used: median 2.0% (0-15.5%) for the TNFα-group and 4.0% (0-21.0%) for the non-TNFα group (P<0.05). However, there was no difference in morbidity between the patients who where perfused with TNFα and those who were not. The fasciotomy and melanoma excision wounds created post ILP usually healed without any major complications.
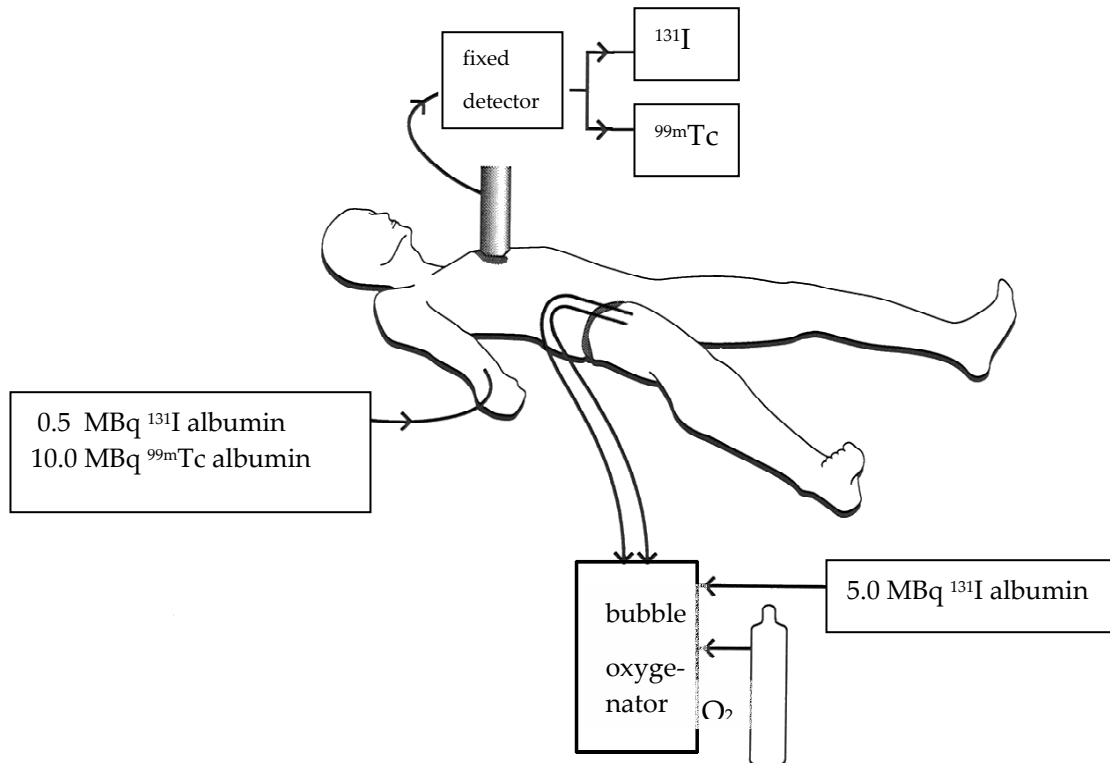
Fig. 3 *Continuous leakage monitoring during isolated limb perfusion with a scintillation detector placed above the heart (with permission of Hoekstra et al. Continuous leakage monitoring during hyperthermic isolated regional perfusion of the lower limb: techniques and results. Regional Cancer Treatment 1992; 4:301-304. Springer Verlag)*

❑ **Discussion**

Creech and Krementz introduced regional isolation perfusion in 1958 as a method to deliver large doses of cytotoxic drugs to the local tumor area while avoiding systemic toxicity.[1] Monitoring of leakage occurring during regional perfusion of the isolated circuit into the systemic circulation has generally been mandatory due
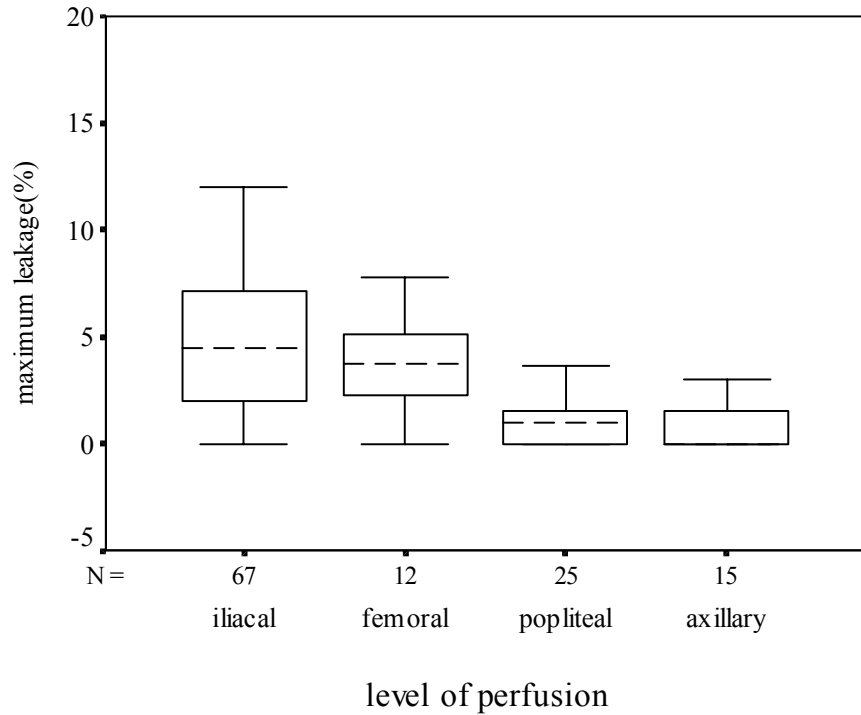
Fig. 4 *Distribution of the maximum leakage and the amount of perfusions corresponding to the level of perfusion (dotted line represents the median value)*

to dose escalation of the chemotherapeutics in the perfusate to levels which would be extremely morbid or lethal if given systemically. Several leakage detection methods are described in the literature. Many groups have monitored the systemic levels of the drug used exclusively through the analysis of intermittent blood samples.[13,14] The flaw in this method is however, that the monitoring is not continuous, and the results take at least 20 minutes before they are known. The surgeon has therefore no possibility for acute intervention if a potentially dangerous amount of leakage of the perfusate is being monitored. A search was therefore initiated for a continuous, real-time method of monitoring. Stehlin was the first to describe a continuous method in as early as 1961 using the radioactive isotope ^{131}I-albumin.[15] In 1989 a hand held gamma detector was introduced by Sardi to be placed between the thighs and precordially in order to record the

74

radioactivity of the ^{131}I-albumin that had leaked into the systemic circulation.[16] However, measurement of the leakage with the handheld detector is accompanied by a method-error, because slight variation in probe positioning gives rise to altered results. Nowadays when a toxic drug like TNFα is being used, accurate leakage measurement is of the utmost importance, since a 1% of TNFα leakage can cause hypotension and a 10% leakage can cause a potentially fatal septic shock like syndrome.[10,11] With the establishment of a stationary scintillation detector placed above the heart, the before mentioned method-error was eliminated.[17]

Despite available detection methods, the data or leakage of the perfusate to the systemic circulation is rarely mentioned in the literature. In a previous article covering the period from 1977 until 1990 at this institution, a median of the maximum leakage of 8.0% (range 0-30%) was reported, with an achieved leakage of less than 15% in 84% of the patients.[7] During this period the drug of choice was melphalan, and a maximum leakage of 15% of melphalan did not prove to cause systemic toxicity.[6] Nevertheless, this median leakage is considered being too high for the currently used treatment schemes which include TNFα. In this period from 1977 – 1990 there was constant experimenting going on with new leakage techniques in order to achieve a minimal leakage. From 1977 – 1982 the perfusions were performed with a low flow rate of 100-150 ml/min with a mean leakage of 7.04 ± 1.04%. On the basis of Fontijne's study a more physiological perfusion was implemented since 1982, and the perfusion pressure was derived from the patients' arterial and venous pressure during the operation.[18] This resulted in an increase in flow rate ranging between 500-900 ml/min. However, this was accompanied with a significant higher mean leakage: 10.43 ± 1.82% (P<0.001). To reduce complications of this increased leakage, the cytotoxic drugs have since 1987 been administered only when the observed leakage is less than 5% over a period of 5 minutes. Consequently, this resulted in a statistically different decrease in leakage to 7.35 ± 0.69% (P<0.001) in patients who were actually perfused with the cytotoxic drugs. When the TNFα perfusions were introduced in the early nineties, no leakage of the perfusion circuit to the systemic circulation was accepted in contrast to the melphalan perfusions. In the current series starting from 1991 the overall median of the maximum leakage was calculated at 2.7% (range 0-21%). This is significantly lower than the period 1977 – 1990 (P<0.05). This difference is according to us based on three aspects. First of all, the previous report is based on 331 iliacal and 55

femoral perfusions and the current series includes 40 axillary and popliteal perfusions from the 119 total perfusions. Univariate analysis showed that the level of isolation is a factor predicative of systemic leakage where the iliacal and femoral levels of isolation had a significant higher chance of leakage than the popliteal and axillary levels of isolation. This aspect has been highlighted before in the literature for which Pace described an optimal method for isolation at the iliac level.[19] His method was to temporary close the common iliac vein during perfusion, for which a mean leakage of 9% was reported. In the Groningen iliacal perfusion technique the external iliac, obturator, hypogastric and the collateral veins are temporary closed.[12] Secondly, since the early nineties, the very toxic cytokine TNFα is increasingly being used in the perfusion setting since it has been proven to be very effective in limb salvaging for STS.[8,20] These cases involve delivery of TNFα levels to the tumor that are approximately 15-20 times the maximally tolerated systemic levels.[4] If there is significant leakage (over 10%), the resultant systemic complications could be fatal. However, Stam *et al.* recently published a report in which a leakage of up to 65% TNFα into the systemic circulation only caused a hypotension which was easily corrected with either fluid administration or dopamine treatment for 2 days.[21] Interestingly, since the introduction of TNFα there has been a significant decrease in leakage compared to when melphalan is used alone (P<0.05). It is our opinion that this decrease in leakage is based upon the surgeons and perfusionists awareness of the added risk of TNFα and not based upon the pharmacokinetics of TNFα.

The third reason for a decrease in leakage is attributed to the decrease in flow rate as described by Sorkin *et al.* in 1995.[22] As mentioned before, since the early eighties at the Groningen institution a high flow rate was used according to Fontijne.[18] But Sorkin observed a decrease in leakage from 12.5% to 2.3% when the flow rate was decreased from 869 ml/min to 286 ml/min. Moreover, Allen reported that a 20% decrease in flow rate will reduce the leakage from the extremity to the general circulation by 50%.[23] This is a very important observation since a sufficient flow rate is required to maintain physiological blood gas values. But the flow rate should however not reach levels causing raised venous pressure which would subsequently lead to an increase in regional toxicity and increased systemic leakage. In our institution a flow rate between 400 and 500ml/min was therefore adapted for this purpose. Just recently a new technique has been implemented

76

where the flow rate is based on the limb volume and is expected to reduce the leakage even further. It is interesting to note that even though the leakage rate in the latter years has decreased, we did not notice a decrease in morbidity between the patients from the two time periods. If there is a substantial leakage, the order of events to minimize the leakage is as follows: First of all, the venous tourniquet is re-applied. If this does not result in a decrease of leakage the systemic pressure is increased to normal pressure values using dopamine and/or bringing the patient into a Trendelenburg position. If the leakage is still unacceptable, the flow rate is subsequently decreased. If the measures taken have no effect on minimizing the leakage, the procedure is terminated without perfusing the limb with the cytotoxic drugs. Klaase *et al.* reported in 1993 a cumulative systemic leakage of 0.9% (95% confidence interval) for their series of 438 perfusions performed at their institution.[5] However, to our knowledge, their leakage detection method differed considerably to the one used in this institution. Their method consisted of a small systemic calibration dose and a higher limb dose of ^{99m}Tc-albumin to measure the leakage. This compared to our method where we use a small calibration dose of ^{131}I -albumin and a higher dose of ^{131}I-albumin for the limb. ^{131}I-albumin has a longer half life than ^{99m}Tc-albumin (8 days compared to 6 hours respectively), which results in a longer circulation of the isotope in the systemic circulation and therefore enhances steady measurement.[24] In our setting, the use of ^{99m}Tc-albumin serves as a control for correct measurement: because its registration is not leakage depended, the time-curve should display a decrease in radioactivity based on the half-life ($t_{1/2}$) time of ^{99m}Tc-albumin. Should an unintentional displacement of the detector occur, then this is readily reflected in a deviation from the ($t_{1/2}$) curve. When only ^{131}I-albumin was used, such a displacement of the detector might be wrongly interpreted as a change in leakage.

In summary, even under the most optimal conditions it is not always possible to achieve total limb isolation when perfusing at the iliacal or femoral level. Using our technique of monitoring, leakage is readily detected and seems to be superior to the technique using ^{99m}Tc-albumin alone. Nowadays the leakage from the isolated circuit into systemic circulation has significantly declined compared to the days when melphalan was the sole drug used. This is due to the increased awareness of surgeons for the very toxic TNFα, which has caused them to operate more

cautiously. The flow rate regulation in the ILP circuit and optimal regulation of the systemic blood pressure have also been major contributors to this improvement. It is therefore possible to achieve a leakage rate of 5% or less.

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80

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Editorial

Isolated Limb Perfusion in the Management of Patients with Recurrent Limb Melanoma: An Important but Limited Role

John F. Thompson and Johannes H. W. de Wilt

Isolated limb perfusion (ILP) was introduced into clinical practice in the mid-1950s, based on the same principles that had been applied to develop extracorporeal cardiopulmonary bypass a few years earlier. The concept was simple. By temporarily isolating the vasculature of a limb, high cytotoxic drug concentrations could be achieved without producing serious systemic side effects. Early studies using hyperthermic ILP with melphalan for limb melanoma produced impressive results, with overall response (OR) rates of around 80% and complete response (CR) rates for measurable limb disease of 30%–50%.[1] More recent reports indicate that CR rates exceeding 50% are now obtained in most melanoma treatment centers where ILP is undertaken.[2] Even higher CR rates have been reported

when tumor necrosis factor (TNF) has been used with melphalan.[3] Apart from the generally unacceptable option of amputation, no other form of treatment (systemic, regional, or local) achieves response rates that are consistently as high as those able to be obtained by ILP. It provides results far superior to those able to be achieved by any form of systemic chemotherapy, for example, where OR rates exceeding 20% are uncommon and CR rates rarely exceed 1%–2%. However, despite the clearly demonstrated effectiveness of ILP for limb melanoma, it is a technique still used in only a small number of centers worldwide. The principal reason for this paradoxical situation is that, although the procedure is elegantly simple in concept, in practice it is a surgical tour-de-force – technically complex and demanding, labor intensive, time consuming, and costly. It has become clear that if ILP is to be effective and safe, meticulous attention to every aspect of the procedure is essential, with comprehensive monitoring of all parameters that might influence efficacy and outcome. These considerations have meant that the use of ILP, both for melanoma and soft tissue sarcoma, has been largely restricted to a

few tertiary referral centers with appropriate academic staff and sufficient resources to undertake ILP as a clinical research endeavor. The control and monitoring of systemic drug leakage, as described in the paper by Daryanani et al.[4] in this issue of *Annals of Surgical Oncology*, represents just one of the many technical challenges which must be overcome if ILP is to be performed with safety. Because of its complexity, cost, and potential morbidity, the objectives of ILP and the indications for its use must be very clearly understood. It was shown to have no effect whatsoever on survival in the large prospective EORTC-WHO-NAPG trial[5] comparing standard surgery plus adjuvant ILP with surgery alone for patients with melanomas ≥1.5 mm thickness. Nor has it ever been demonstrated to influence survival when used as a therapeutic procedure in the presence of macroscopic limb disease. ILP can nevertheless generate considerable morbidity, depending upon such factors as the type and dose of chemotherapeutic agents used, adequacy of oxygenation, level of hyperthermia, perfusate flow rate, and duration of perfusion. Potential complications, including damage to blood vessels, nerves, and muscles,[6] need to be considered before ILP is recommended. Recurrence or in-transit metastasis confined to a limb is reported to occur in 5%–8% of melanoma patients. Satisfactory but much simpler treatment options than ILP are often available for this small group of patients. In some cases, simple surgical excision of one or two small in-transit metastases will be all that is required. If disease is more extensive but superficial, techniques such as cryotherapy, diathermy curettage, laser treatment, radiofrequency ablation, or direct cytotoxic drug injection into tumor

nodules can be effective. For larger and more deeply placed lesions, local radiotherapy can be used. Recent studies have demonstrated that electroporation therapy, in which an electric field is applied across a tumor nodule via a circular array of needle electrodes after injection of a cytotoxic agent into it, is also effective in many cases. Thus, in patients who have limb recurrences of melanoma which are small and few in numbers, easily removed by surgical excision, or able to be treated by one of the other simple methods outlined above, it is difficult to justify treatment by ILP. In some (but not all) patients, further local or in-transit recurrences will become apparent later, but often these are able to be dealt with by simple means once more. There remains, however, a group of patients with disease that is too advanced or too widespread within the limb to be able to be treated by these methods, and it is in this situation that isolated regional chemotherapy using a technique such as ILP is indicated. Acknowledging the effectiveness of ILP, but recognizing the limitations and risks imposed by its technical complexity, simpler and safer regional chemotherapy techniques have been sought. Direct intra-arterial infusion of cytotoxic agents was simple, but although an enhanced first-pass effect of the drugs on the tumor was achieved, CR rates were unimpressive and systemic drug toxicity was a major problem. Another alternative technique was described as tourniquet infusion but, again, response rates were low and systemic drug effects were inevitable because only very brief occlusion of venous outflow from the limb was achieved.[7] At the Sydney Melanoma Unit, we introduced in 1992 an alternative technique that we called isolated limb infusion (ILI). This has produced results generally comparable to those achieved

by ILP, but without requiring complex equipment, many staff, and long periods of operating time. We have found the technique to be well tolerated, even by frail and elderly patients, allowing effective palliative treatment to be given to individuals who would not be considered fit for treatment by conventional ILP. Details of the ILI procedure have been reported elsewhere,[8] but it is, in essence, a very low flow ILP performed without oxygenation via standard radiological catheters (6–8 FG). These are inserted percutaneously in the radiology department prior to the actual ILI procedure in the operating room, which involves only 20–30 minutes of drug exposure. Systemic drug leakage is minimized by routine use of a pneumatic tourniquet and a generous washout of the limb vasculature prior to deflation of the tourniquet and removal of the catheters. These maneuvers virtually eliminate the risk of systemic side effects, with systemic melphalan leakage rarely exceeding 1%–2% (calculated from measurements of systemic melphalan concentrations by high performance liquid chromatography). The results of ILI for melanoma in terms of OR and CR rates have been similar to those achieved by conventional ILP, and it has been found easily possible to repeat the procedure if a satisfactory response is not achieved after an initial ILI or if recurrence in the limb occurs later. Although the effectiveness of isolation techniques used during conventional ILP have improved over the years, as the article by Daryanani et al.[4] reports, and a decrease in systemic drug leakage and associated systemic complications has been achieved as a result of this, the procedure remains complex and costly and can produce considerable regional morbidity. Because ILP has no effect on survival, its objective must be to achieve local disease control in a limb. However, it should only be used if it is likely to produce better or more long lasting results than other forms of locoregional treatment that are simpler, cheaper, less invasive, and safer.

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Chapter V

❑ Pregnancy and early stage melanoma

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85

❑ Introduction

Melanoma is often diagnosed in young adults and a significant number of these patients are women in their reproductive phase of life. The real incidence of melanoma during pregnancy is unknown. Smith and Randall reported in 1969 an incidence of 2.8 per 1,000 deliveries.[1] The influence of pregnancy on the prognosis of melanoma has been a controversy ever since 1951 when Pack and Scharnagel reported ten pregnant patients with malignant melanomas of which five died within 30 months after diagnosis.[2] Since then numerous case reports have also been presented showing a worse prognosis compared to non-pregnant women and the dilemmas in treatment policies.[3,4] But in 1985 the World Health Organization melanoma program initiated a retrospective study of melanoma in relation to pregnancy which showed that there were no survival differences between pregnant and non-pregnant Stage I patients after correction for tumor thickness.[5] Thus the literature is confusing, and reports on the behavior of the melanoma are contradictory.

It has been hypothesized that the frequency and prognosis of melanomas in women is influenced by hormonal or reproductive factors. However previous studies have failed to prove that the presence of hormonal receptors in human melanoma cells carries a poorer prognosis of the disease.[6-8] Traditional quantitative analyses suggest that a proportion of the melanoma cells carry a type II oestrogen receptor, but the absence or presence of this receptor does not appear to affect the individual outcome of the patient.[8] This has been underlined in recent reports in which the relation between the use of oral contraceptives and the prognosis could also not be proven.[9-11]

The purpose of this present study is to report our single institution's experience with melanoma arising during pregnancy. Specific goals of the study are to determine the disease free survival (DFS) and overall survival (OS) rates of this group of patients during a long term follow up period. Furthermore, the impact of Breslow thickness, ulceration, vascular invasion, histologic subtype and location of the tumor on the DFS and overall survival was studied.

❑   **Patients and Methods**

Between 1965 and 2001, a total of 2567 patients were diagnosed and treated for a cutaneous malignant melanoma at the University Medical Center Groningen. The population consisted of 991 male patients (39%) and 1576 female patients (61%). For the purposes of this study, 46 female melanoma patients with a median age of 30 (range 18-46) years were identified to be pregnant and diagnosed with an American Joint Committee on Cancer (AJCC) TNM Stage I/II melanoma (Table 1)[12]. This pregnant group was compared to a non-pregnant control group. This age-sex control population was selected of those Stage I/II cutaneous female melanoma patients between 16 and 46 years of age who were not pregnant at time of diagnosis. This subgroup consisted out of 368 female non-pregnant Stage I/II melanoma patients with a median age of 36 (range 17-45) years. The clinical data retrieved from the Groningen melanoma database included age at diagnosis, site of the primary tumor, and stage at diagnosis. All histological slides were reviewed at our institution. The histopathological characteristics recorded included the histologic subtype of melanoma (superficial spreading, nodular or unknown), tumor ulceration, Breslow's depth of invasion (in millimeters) and the presence or absence of vascular invasion.

Treatment

All patients were treated according to the guidelines of the Dutch Melanoma Working Party.[13] Most patients who were referred to our institution underwent a diagnostic excision of their primary lesions elsewhere. The patients were referred for further treatment consisting mostly of wide excision only. The sentinel lymph node biopsy was performed since 1995 in 6 patients, all of which were postpartum. Fifteen (33%) patients in the pregnant group (all postpartum) received an adjuvant hyperthermic isolated limb perfusion with melphalan compared to 144 patients (39%) in the non-pregnant group. The adjuvant limb perfusion was in the past part of an institutional protocol or part of the EORTC study on adjuvant limb perfusion in Stage I melanoma.

Staging	T classification[†]	Thickness[*]	Ulceration Status
	T1A	≤ 1.0 mm	w/o ulceration
1A			
	T1B	≤ 1.0 mm	with ulceration
1B			
	T2A	1.01 – 2.0 mm	w/o ulceration
	T2B	1.01 – 2.0 mm	with ulceration
2A			
	T3A	2.01 – 4.0 mm	w/o ulceration
	T3B	2.01 – 4.0 mm	with ulceration
2B			
	T4A	>4.0 mm	w/o ulceration
2C	T4B	>4.0 mm	with ulceration

[†]*2002 AJCC TNM classification;* [*] *Thickness according to Breslow*
mm: millimeter; w/o: without

Table 1. *2002 AJCC TNM melanoma staging system[12]*

Follow up

All patients were regularly followed in the outpatient clinic for regional and/or distant failures. The follow up scheme did not include lab controls, only yearly chest x-rays. Regional failure was defined by the histological or cytological confirmation of tumor cells at the site of the primary tumor and/or along its lymphatic drainage pathway to the first lymph node basin. Distant failure was defined by radiological detection of suspected lesions by means of computer tomography (lungs, brain), ultrasound (liver) and bone scintigraphy. Histological confirmation of distant failure was not obtained in all cases. Appropriate treatment of recurrences was performed depending of the type and site of recurrence. No patient received any kind of adjuvant systemic treatment.

88

Statistics

The software package SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL) was used for statistical analysis. To compare the two groups in Breslow thickness the Mann Whitney U test was used. Disease free survival was defined as the time between the date of diagnosis and the date of first recurrence. The follow up time was defined as the time between the date of diagnosis and the date of disease-specific death or last visit to the outpatient clinic. The Kaplan-Meier, Cox regression and logistic regression analysis tests were performed in order to identify the risk factors and to calculate the 10-year DFS and 10-year overall survival curves with the date of diagnosis, first recurrence or death acting as end-points. To compare the survival distributions between the pregnant and the non-pregnant group a log rank test was used. Potential prognostic factors were calculated using a univariate and multivariate analysis. A *p*-value less than 0.05 was considered to be significant.

❑ **Results**

Clinical data

Data on 610 consecutive women of childbearing age with cutaneous melanomas were analyzed. Fifty seven patients were pregnant at the time of diagnosis (46 Stage I/II, 8 Stage III and 3 Stage IV). The number of Stage III and IV patients was not sufficient for statistical analysis and therefore not considered. The age-sex non-pregnant control group consisted of 368 of the total 610 childbearing female patients who had sufficient clinical and pathologic available. The clinical variables of the Stage I/II pregnant patients and the non-pregnant patients are presented in Table 2. The median follow up time was similar for both the pregnant and non-pregnant groups. The median follow up time for the pregnant patients was 106 (range 3–260) months and 109 (range 1–356) months for the non-pregnant patients. Extremity primary melanomas predominated in this group of patients. For the pregnant group, 36 patients (78%) had extremity melanomas with 28 patients originating from the lower extremities (61%). Seven patients had trunk (thorax, abdomen and back) melanomas (15%) and 3 patients had head and neck melanomas (7%). For the non-pregnant group the percentages were respectively

Total Group	Pregnant group		Non-pregnant group		Logistic regression	
	N	%	N	%	DFS	OS
Patients (N = 414)	46	11	368	89		
Median Age (range)	30 (18–46) yrs		36 (17 – 45) yrs		*ns*	*ns*
Upper extremity	8	17	51	14		
Lower extremity	28	61	229	62	*p<0.004*	*ns*
Trunk	7	15	66	18	*ns*	*ns*
Head and Neck	3	7	22	6	*ns*	*ns*

ns = not statistically significant; DFS = Disease free survival; OS = Overall survival

Table 2. *Demographic data of the pregnant and non-pregnant group and logistic regression analysis on DFS and OS*

76%, 18% and 6%. See Table 2 for a summary of the primary tumor locations. Patients were staged according to the 2002 AJCC TNM system as presented in Table 3.

Histopathological Data

All primary tumors were histologically classified according to histologic subtype, Breslow thickness, vascular invasion and tumor ulceration and are summarized in Table 4. There was no significant difference between the two groups in histologic subtype. The majority of the tumors were superficial spreading melanomas, 43% in the pregnant group compared to 48% in the non-pregnant group. There was also no difference in the frequency of the nodular type melanoma (20% pregnant vs. 13% non-pregnant, *ns*). In the pregnant group the median Breslow thickness was 2.0 mm compared to 1.7 mm in the non-pregnant group (*ns*, p=0.119 Mann

90

Whitney U test). In the pregnant group the incidence of blood vessel invasion was 9% compared to 2% in the non-pregnant group (*ns*, p>0.05). The number of tumors that were classified as ulcerating was also comparable in both groups.

Disease free and Overall Survival

For the Stage I melanomas the 10-year DFS was calculated to be 88% for the pregnant group compared to 86% for the non-pregnant patients (p=0.6541). The 10-year OS was respectively 94% and 90% (p=0.6977). Patients who had Stage II disease and were pregnant had a 10-year DFS of 67% compared to 73% for the Stage II non-pregnant patients (p=0.7322). The OS for the Stage II patients was respectively 82% and 81% (p=0.7162) (see Fig. 1 and 2). Logistic regression analysis showed that increasing Breslow thickness (p<0.005) and the presence of ulceration (p<0.036) significantly led to a higher mortality. No significant effect of vascular invasion, tumor type, tumor location or pregnancy could be detected on the overall survival. Logistic regression analysis with DFS as dependent variable revealed that the length of the DFS is related to increasing Breslow thickness (p<0.006), ulceration (p< 0.010), location of the tumor (extremity melanoma) (p<0.004), histologic subtype (nodular melanoma) (p<0.002) but not vascular invasion (*ns*, p=0.076) (Table 2 and 4).

Staging*	Pregnant		Non-pregnant	
	N	%	N	%
1A	11	24	113	31
1B	13	28	96	26
2A	13	28	94	26
2B	5	11	45	12
2C	4	9	20	5

* See Table 1

Table 3 *Staging of the pregnant and non-pregnant patients according to the 2002 TNM AJCC system[12]*

In this model 30% of the variance of the DFS could be explained by the model. Although pregnancy showed no significant effect on the DFS, it did reveal a trend towards a longer DFS in the non-pregnant patients.

❏ Discussion

The possible influence of hormonal factors on the pathogenesis and biologic behavior of melanoma have intrigued scientists and clinicians on the basis of clinical or anecdotal reports suggesting a detrimental influence of concurrent pregnancy. The earlier hypothesis was that during pregnancy there was an increase in melanin stimulating hormone (MSH), oestrogen and progesterone which in turn lead to increased pigmentation and size of benign naevi and even possible malignant transformation.[14] But recent reports have denied the expression of estrogen receptors on human melanoma cells that affect the outcome of the disease.[7,15] In a large cohort study of sub-fertile women, Venn et al. reported no evidence of an increase in melanoma risk in women who received exogenous hormones for in vitro fertilization, compared to a control group.[16] Thus there are to date no clear indications that exogenous hormone stimulation or hormonal changes during pregnancy stimulate the growth of the primary tumor or of potential micro-metastases. Furthermore, there is also no evidence that exogenous hormones, such as oral contraceptive medication, are contraindicated after treatment for a melanoma.

In our research the effect of pregnancy on melanoma progression was determined by comparing the 10-year DFS and overall survival in female patients of childbearing age, pregnant vs. non-pregnant, at the time of diagnosis of clinically localized melanoma. Our study shows no difference in the 10-year DFS between the pregnant group and the non-pregnant group (Stage I 88% vs. 86%, Stage II 67% vs. 73%) and no significant difference in the 10-year overall survival (Stage I 94% vs. 90%, Stage II 82% vs. 81%) (Figs.1 and 2). Several large international studies including the report from the World Health Organization have attempted to answer this same question about a decade ago using multivariate regression analyses.[5,17-21]

	Pregnant group		Non-pregnant group		Logistic regression	
	N	**%**	**N**	**%**	**DFS**	**OS**
Histologic subtype					$p<0.002$	ns
Unknown / other	17	37	145	39		
SSM	20	43	177	48		
Nodular	9	20	46	13		
Breslow thickness (mm)*					$p<0.006$	$p<0.005$
0 – 0.75	12	26	116	32		
0.76 – 1.50	14	30	122	33		
1.51 – 4.0	14	30	97	26		
> 4.0	6	13	33	9		
Vascular invasion					ns $(p=0.076)$	ns
Absent	39	85	328	89		
lymphatic	2	4	15	4		
Blood vessel	4	9	8	2		
Unknown	1	2	17	5		
Ulceration					$p<0.010$	$p<0.036$
Yes	10	22	84	23		
No	36	78	284	77		

*SSM = Superficial Spreading Melanoma; * sum of percentages may not add up to 100% due to rounding; DFS = Disease free survival, OS = Overall survival, ns = not statistically significant*

Table 4 Histopathological data of the primary tumor and logistic regression analysis on DFS and OS

DFS pregnant vs non-pregnant

p=0.6541

p=0.7322

Legend:
- Pregnant Stage I
- Non Pregnant Stage I
- Pregnant Stage II
- Non pregnant Stage II
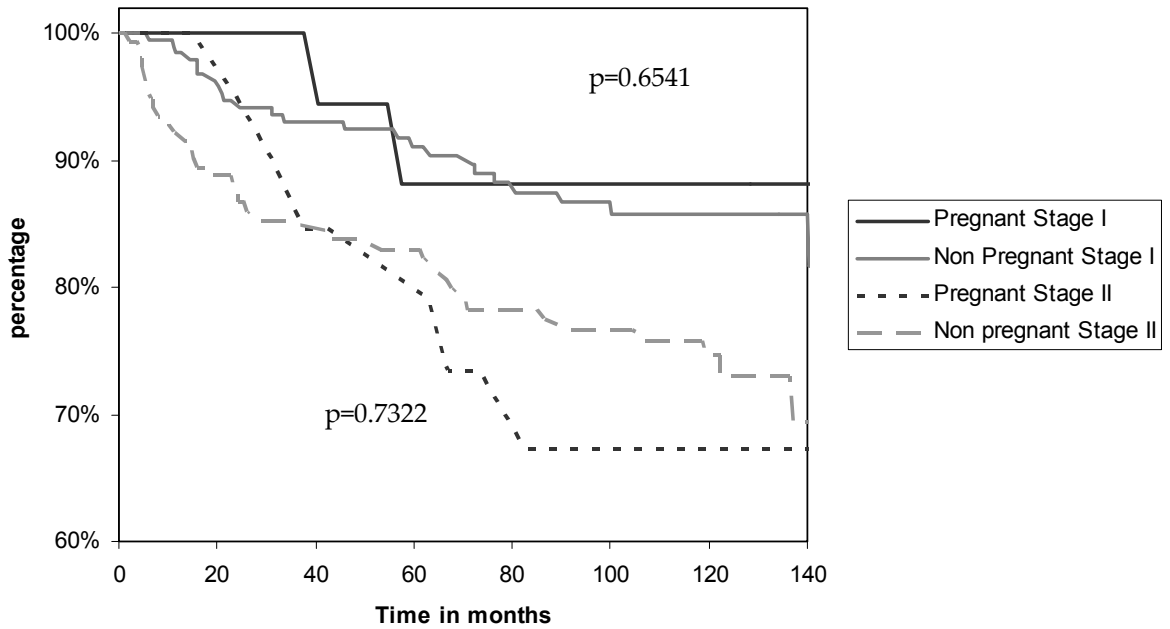
Fig 1. Disease free survival pregnant vs. non pregnant for Stage I and Stage II AJCC

Data from these studies suggest that pregnant women with the previous AJCC staging system Stage I and II melanoma (primary site only, with any tumor thickness) did not show any survival or DFS difference if treated promptly and appropriately. However the majority of these studies fail to mention the length of the follow up period. Two similar reports published by Duke University showed a significant shorter DFS in the pregnant group without any effect detrimental on overall survival.[22,23] They reported that 51% of the pregnant patients and 68% of control patients remained disease free at 10 years. But although pregnancy showed no significant effect on the DFS in this series, it did possibly reveal a longer DFS in the non-pregnant patient.

94

Overall survival pregnant vs non-pregnant
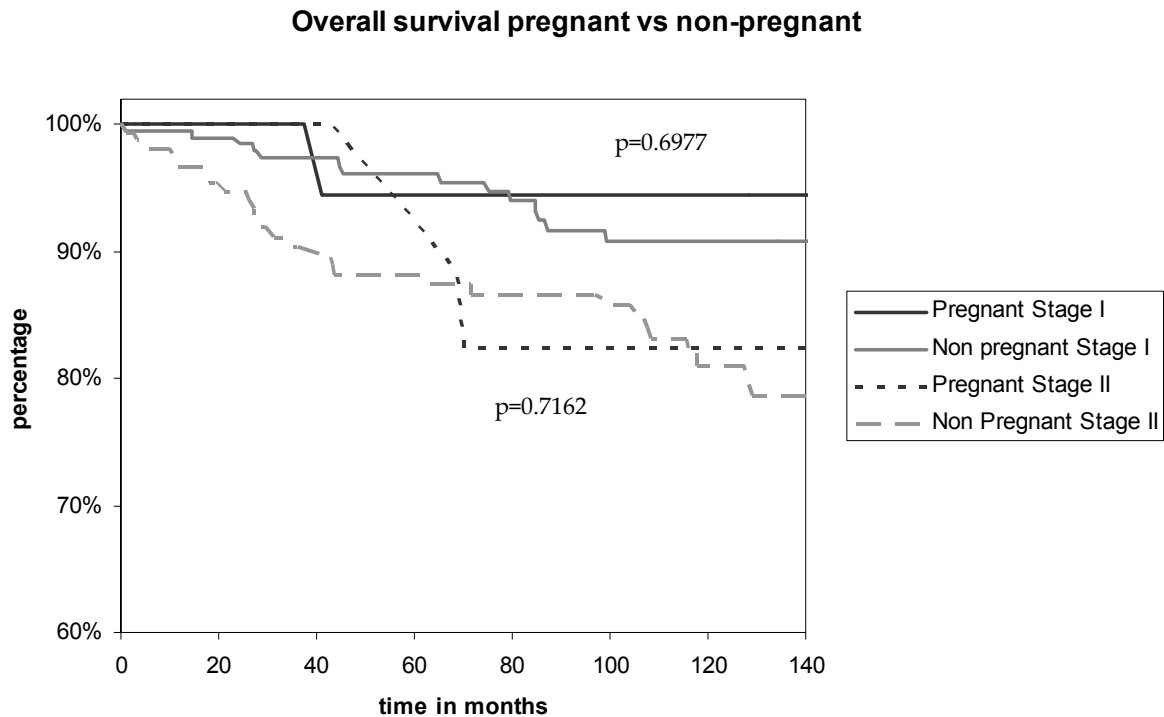
Fig 2. Overall survival pregnant vs. non-pregnant for Stage I and Stage II AJCC

An interesting phenomenon is the slightly increased median thickness of the primary melanomas in the pregnant patients compared to the non-pregnant group in this study and other studies.[5,19,23] In this series we noted thicker primary tumors (median 2.0 vs. 1.7 mm, p=0.119) in the pregnant patients, but we failed to show a significant effect on the DFS or OS. Overall the general thought that naevi enlarge or that new naevi arise during pregnancy. The hypothesis is that salient signs of early melanoma, growth and change, may be initially misinterpreted as physiologic pregnancy-related changes leading to a change in diagnosis.[24] However Pennoyer et al. recently reported that only a small proportion of naevi (6%) showed a change during pregnancy.[25] Further research is necessary to confirm these results in a larger study population. If this is confirmed, this would mean that the advice to pregnant women should be the same as to non pregnant women

95

that timely biopsy of all suspicious naevi should be performed and not deferred to after delivery.[24]

Our study also focused on the effect of Breslow thickness, ulceration, vascular invasion, histologic subtype and location of the tumor on the DFS and overall survival in the pregnant patient and in the non-pregnant group. In both the pregnant and in the non-pregnant group there was no difference in the presence of ulceration, vascular invasion or location of the primary tumor. Primary tumor locations were comparable with the literature where extremity melanomas predominate in this group of patients.[26] Logistic regression analysis on mortality and DFS showed that increasing Breslow thickness (OS: p<0.005, DFS: p<0.006) and the presence of ulceration (OS: p<0.036, DFS: p<0.010) were identified as independent prognostic factors. This was previously reported in the literature and is the foundation on which the 2002 AJCC TNM classification is based.[12] Furthermore logistic regression analysis on DFS also showed that extremity melanoma (p<0.004) and nodular type melanoma (p<0.002) are prognostic unfavorable factors of Stage I/II melanoma. Although we could not confirm vascular invasion as being a prognostic factor, we did establish a trend towards a poorer prognosis (p=0.076).

In conclusion, based on these results and a critical review of the literature, pregnancy at the time of diagnosis of clinically localized invasive melanoma is not a risk factor for disease progression. Pregnant patients may present with thicker melanomas and pregnancy may decrease disease free survival but neither melanoma thickness nor disease free survival were significantly different between pregnant and non-pregnant patients in this study. In melanoma patients pregnant at the time of diagnosis, survival is still dependent on the tumor thickness and the absence or presence of ulceration. Patients should therefore be counseled regarding these factors and not the pregnancy.

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98

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EDITORIAL

Management of Patients with Melanoma who Are Pregnant, Want to Get Pregnant, or Do Not Want to Get Pregnant

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The number of pregnant women who are newly diagnosed with malignant disease annually in the U.S. is approximately 1 per 1000 population.[1] Melanoma, breast carcinoma, cervical carcinoma, leukemia, and lymphoma are the most common types of malignant disease that arise during pregnancy.[2,3] The incidence of melanoma is rising at a rapid rate, and it is projected that 1 in 68 Americans born in the year 2002 will develop an invasive melanoma during their lifetime. Melanoma is the sixth most common malignancy in women. It is the most common malignancy in women ages 25–29 years, and it is associated with a younger age at the time of presentation.[4] These trends suggest that approximately 35% of women with melanoma are of

100

childbearing age.[2,5] In this issue of *Cancer*, Daryanani et al. provide further evidence that pregnancy does not have an adverse effect on prognosis in patients with melanoma.6 The information from this single-institution study is valuable; however, the management of patients with melanoma with respect to pregnancy and hormonal contraception remains a challenge. The purpose of this editorial is to discuss the management of these patients and to enhance the information provided by Daryanani et al.[6] It is clear that we will encounter increasing numbers of women of childbearing age who may be pregnant at the time of diagnosis of melanoma, who may desire to become pregnant after the diagnosis, or who may desire to use oral contraceptive pills to prevent pregnancy after a diagnosis of melanoma. In clinical practice, how these scenarios are approached may vary from institution to institution and from physician to physician. This is simply a reflection of the historic controversy that has surrounded the effect of pregnancy and hormones on melanoma and the appropriate management of melanoma in pregnant women and in women of childbearing age. Although no rigorous, randomized, prospective trials exist to answer all of these questions definitively, a wealth of epidemiologic data and controlled clinical studies provides consistent insight into optimal management approaches.

Pregnancy at the Time of Diagnosis
Numerous well controlled studies, such as that by Daryanani et al., have provided strong evidence that the clinical course, prognosis, and overall survival of pregnant women with melanoma (American Joint Committee on Cancer [AJCC] Stage I–II) is similar to that in nonpregnant women.[2,3,5–9] The prognosis of pregnant women with melanoma still

is dependent primarily on tumor thickness and ulceration status.[10] Some studies have shown that thicker lesions are associated with pregnancy, presumably as a result of delayed diagnosis.[8] Early detection is critical, and prompt biopsy of suspicious lesions is important and should not be deferred until after pregnancy. The earliest sign of a melanoma is a change in the size, shape, or color of a lesion. The earliest symptom is persistent itching of a lesion.[11] Biopsies and wide local excisions can be performed safely during pregnancy. Minor variations in logistics and techniques easily can accommodate the recommendations of the patient's obstetrician. For example, some of our obstetricians recommend recording fetal heart rate before and after the mother undergoes a wide local excision.

Can Pregnant Patients Undergo Sentinel Lymph Node Biopsy Safely?
Sentinel lymph node (SLN) mapping and biopsy represent one of the most significant advances in the management of patients with melanoma during the past decade. The most powerful prognostic factor related to recurrence and survival in patients with clinically localized melanoma is the status of the SLN.[12] We offer SLN biopsy at our institution to relatively healthy, nonpregnant patients with melanomas measuring ≥ 1.0 mm or < 1.0 mm with other adverse factors. This technique uses preoperative intradermal injection of 99mTc-sulfur colloid (1–3 millicuries [mCi]; CIS-US, Inc., Bedford, MA) and intraoperative, intradermal injection of isosulfan blue dye (Lymphazurin 1%; Hirsch Industries, Inc., Richmond, VA) around the intact tumor or biopsy site. Lymphatic mapping and SLN biopsy are performed with the aid of a hand-held gamma counter and visualization of blue lymph nodes. Fetal doses < 100 milli-

grays (mGy) of radiation do not increase the incidence of fetal malformation. The Society of Nuclear Medicine recommends a pregnancy test in patients prior to any procedure that would expose a fetus to > 50 mGy. The dosages and radiopharmaceuticals used for lymphatic mapping deliver whole fetal doses of < 5 mGy. Therefore, these radiopharmaceuticals at dosages used for lymphatic mapping are not contraindicated in pregnant patients and carry negligible risk.[13–15] The safety of isosulfan blue dye in pregnancy poses additional questions. The rate of allergic reactions to the dye reportedly is as high as 2%, and the incidence of life-threatening anaphylactic reaction requiring vigorous resuscitation is reported at 0.7–1.1%.[16,17] Due to the risk of this rare but potentially catastrophic event of anaphylaxis in pregnant patients, we now use radiocolloid alone for SLN biopsy in pregnant patients at our institution. Alternatively, in women who are in middle to late pregnancy and who have undergone narrow excision of their primary melanoma with clear histologic margins, definitive wide local excision and SLN biopsy with radiocolloid and blue dye may be delayed until after delivery, with close clinical follow-up for the duration of the pregnancy.

Implications for the Fetus

What about the risk of metastasis to the placenta and fetus? Fortunately, placental and fetal metastasis from maternal malignant disease is an exceptionally rare event.[18] However, melanoma is the most common type of malignancy to metastasize to the placenta and fetus, representing 30% of placental metastases and 58% of fetal metastases. Approximately 19 patients with melanoma placental metastasis have been reported, with maternal death in all patients: 80% within 3 months of delivery. Five patients with fetal metastasis have been reported, with four resulting in death. Therefore, although these occurrences are exceedingly rare, pregnant patients with melanoma at our institution are counseled, the placenta is sent for histologic evaluation, and the neonatal service is notified.

Pregnancy After Diagnosis

Although there is no clear evidence to suggest that prior, present, or subsequent pregnancy adversely affects the prognosis of patients with melanoma, there are no standard, defined guidelines for patients who desire to become pregnant after diagnosis and treatment of melanoma. Recommendations regarding the length of time to wait after a diagnosis vary from physician to physician, and some physicians recommend against getting pregnant. Significant proportions of patients with localized disease (AJCC Stage I–II) harbor occult disease and eventually develop recurrences. It is not completely predictable who will and who will not develop recurrent disease. Many survival models demonstrate that tumor thickness is the single most important predictor of a probability of cure in patients who do not undergo SLN biopsy. Although approximately 50% of recurrences develop by 3 years in patients with thick lesions, late recurrences occur in some patients, even with thin lesions, longer than a decade after treatment.[19] If recurrence develops during pregnancy, then it may be disastrous both medically and emotionally, potentially altering treatment options and with a risk of placental and fetal metastasis, although these are rare. The use of chemotherapy, radiation therapy, radiodiagnostic tests, and general anesthesia for extensive surgical procedures may have harmful effects on the fetus/ newborn. Our recommendation about how long to wait

before becoming pregnant after a diagnosis of melanoma is on a case-by-case basis, depending primarily on the risk of recurrence (tumor thickness and tumor stage), age of the patient, and desire to become pregnant. There is no exact answer, and our approach is to educate patients completely about the risks to help them make informed decisions. At our institution, patients typically wait 0–5 years, depending on the aforementioned factors. For example, a woman age 40 years with a melanoma measuring 0.3 mm in thickness has only an approximate 1–3% risk of recurrence within 5 years, and we would not necessarily advise her to wait if she desires to become pregnant and finds this an acceptable risk. Alternatively, a woman age 21 years with a melanoma measuring 4.0 mm thick generally would be advised to wait for 3–5 years. Each patient is approached individually, with the patient ultimately making her own informed decision. If the patient does become pregnant, then self-examination of the skin and lymph nodes on a monthly basis is stressed. Although controlled studies consistently demonstrate no statistically significant difference in survival for melanoma in pregnant patients compared with non-pregnant patients, the tragedy of a child losing their mother soon after birth is highlighted by clinical examples and case reports. Merkus et al. illustrated this point in their description of a couple who consulted the gynecologist repeatedly because of a primary fertility disorder.[20] A few days after the woman started the hormonal treatment that preceded the in vitro fertilization procedure, she noted swelling in the right groin area for which she consulted a surgeon. It was found that the swelling was a metastasis from a primary cutaneous melanoma that had been excised from her leg 6 months previously. Her first in vitro fertilization treatment resulted in a pregnancy, which ended with the birth of a healthy boy. However, the placenta showed melanoma metastases, and the mother died 2 months after the birth of her son. Although recurrence and survival may not have been affected directly by pregnancy or hormonal treatment, the tragedy of this situation is readily apparent.

The Desire Not to Become Pregnant (Oral contraceptives)
Given the concerns that may face a woman of childbearing age who has been diagnosed with melanoma, what about oral contraceptive use? Many studies with thousands of patients have demonstrated no significant increased risk for the development of melanoma associated with oral contraceptive use, including duration of use, age started, years since first or last use, or current use.[21] There also is no evidence showing that hormone replacement therapy for menopause plays an etiologic role in the development or recurrence of melanoma. Because a very small risk of contraceptive or replacement hormones on recurrence has not been excluded in a prospective trial, some patients and/or physicians may choose not to accept even a theoretical risk and, instead, choose nonhormonal contraception or menopause symptom relief.

Conclusions
From previous studies, including that reported by Daryanani et al. in this issue of *Cancer*,[6] we may conclude that pregnancy or hormone use before, during, or after a woman is diagnosed with melanoma does not appear to influence survival, which provides important information that should simplify the clinical management of these patients. However, generalized

guidelines may not be applicable or appropriate because of patient variability. In clinical practice, this translates into individualized patient management. The patient with melanoma who is diagnosed during pregnancy is managed best by early and continual involvement of a multidisciplinary team with clinical and psychosocial expertise in melanoma therapy.

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Chapter VI

❑   Childhood and juvenile melanoma

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❑ Introduction

The worldwide incidence of melanoma is increasing and despite significant advances in prevention, early detection and treatment, the mortality rate continues to increase.[1-3] Melanoma before puberty is rare, but the incidence increases steadily thereafter in each decade. The steepest trends of increasing incidence are in those aged over 65 and the lowest trends are in the younger age groups. Approximately 1 to 4% of all new melanoma cases occur in patients under the age of 20 years and only 0.3% among those younger than 14 years.[4-6] The highest rate is reported from Australia where 0.3% to 0.9% of the patients are under the age of 15.[7] Spitz nevus, also known as juvenile melanoma or spindle and epithelioid nevus, is a benign tumor that predominantly affects children and adolescents.[8] Due to its histological overlap with cutaneous melanoma, misdiagnoses of Spitz nevi as well as cutaneous melanoma is not uncommon. The diagnosis of melanoma is sometimes even delayed until the patient develops recurrent disease or distant metastases. It is known that genetic susceptibility and chronic exposure to sunlight during childhood are risk factors for developing a melanoma.[7,9] Factors such as the presence of a giant congenital melanocytic nevi, the phenotype of the nevus, xeroderma pigmentosum and dysplastic naevus syndrome are all associated with a greater risk for developing a melanoma. In this study we analyzed the childhood and juvenile melanomas treated at our institution with a special focus on differences in clinical characteristics, prognostic factors, disease free and overall survival in comparison with those of adults.

❑ Patients and Methods

Between 1965 and 2003, a total of 2567 patients were diagnosed and treated for a cutaneous melanoma at the Groningen University Medical Center (GUMC). The population consisted of 991 male patients (39%) and 1576 female patients (61%). During this period, 55 patients (2.1%) of which 29 males (53%) and 26 females (47%), were under the age of 19 years old. Of these 55 patients, six were under the age of twelve (10.9%), and 49 were between 12 and 19 years old (89.1%). Because of the low number of patients under the age of 12 years, they were not considered in

106

the analysis of the prognostic factors. The melanomas were staged according to the 2002 American Joint Committee on Cancer (AJCC) staging system.[10] The clinical and histopathological data was retrieved from the Groningen melanoma database (Table 1 and 2). The clinical data retrieved included gender, age at diagnosis, location of the primary tumor, and stage at diagnosis. The histopathological characteristics recorded included the histologic subtype of melanoma (superficial spreading, nodular or other/unknown), type of cell (spindle, epithelioid or other) Breslow's depth of invasion (in millimeters), tumor ulceration, the presence or absence of vascular and/or lymphatic invasion and mitotic rate per 5 high power fields (per mm²). All histological slides were reviewed at our institution.

Treatment

All patients were treated according to the guidelines of the Dutch Melanoma Working Party.[11] Most patients, who were referred to the GUMC, underwent excision of their primary lesions elsewhere. The patients were referred for further treatment consisting mostly of wide excision only. In certain high risk patients a so-called en-bloc (wide excision plus lymph node dissection) excision was performed. Therapeutic lymph node dissection was performed in patients staged with pathological involved nodes. A sentinel lymph node biopsy was performed since 1995 in 2 patients. Fifteen (5 children and 10 juvenile) patients were subjected to an adjuvant hyperthermic isolated limb perfusion between the years of 1973 and 1984. The adjuvant limb perfusion was till 1988 part of an institutional protocol for which the results have previously been published.[12]

Follow up

All patients were regularly followed in the outpatient clinic for regional and/or distant failures. The follow up included history, physical examination and yearly chest x-rays. Regional failure was defined by the histological or cytological confirmation of tumor cells at the site of the primary tumor and/or along its lymphatic drainage pathway to the first lymph node basin. Distant failure was defined by radiological detection of suspected lesions by means of chest-X-ray, CT (lungs, brain), ultrasound (liver) and/or bone scintigraphy. Appropriate treatment

	Children	Juvenile	Control group
Total Patients	6	49	972
Age median (range)	7 (3 – 10) yrs	17 (12 – 19) yrs	49 (20 – 93) yrs
Male/Female (%)	3(50%) / 3(50%)	26(53%) / 23(47%)	379(39%) / 593(61%)
Extremity	2 (33%)	31 (63%)	594 (61%)
Trunk	2 (33%)	14 (29%)	229 (24%)
Head and neck	2 (33%)	4 (8%)	149 (15%)
Stage 0 (in situ)	1 (17%)	3 (6%)	0
Stage I	1-IA (17%)	11-IA (23%) 5-IB (10%)	214-IA (22%) 200-IB (21%)
Stage II	3-IIB (50%)	9-IIA (18%) 3-IIB (6%) 4-IIC (8%)	177-IIA (18%) 128-IIB (13%) 54-IIC (5%)
Stage III	1-IIIC (17%)	13-IIIB (27%)	25-IIIA (3%) 110-IIIB (11%) 50-IIIC (5%)
Stage IV	0	1 (2%)	14 (1%)

Table 1: *Clinical data and staging[10]*

of recurrences was performed depending of the type and site of recurrence. No patient received any kind of adjuvant systemic treatment.

Control group and statistics

An adult control group was used to construct explanatory models for comparison between the two groups. Statistical methods used included logistic and linear

regression models (multivariate) in order to calculate the prognostic factors determining the disease free survival and overall survival for the adult control

		Children	**Juvenile**	**Control group**
Breslow thickness (mm)	0 – 1.0	2 (33%)	14 (29%)	236 (24%)
	1.01 – 2.0	0 (0%)	19 (39%)	268 (28%)
	2.01 – 4.0	2 (33%)	11 (22%)	301 (31%)
	> 4.0	2 (33%)	5 (10)	167 (17%)
	Median(range)	3.9 (i.s. – 5.0)	1.6(i.s. – 8.0)	2.0 (0.2 – 35.0)
Ulceration	Yes	2 (33%)	20 (41%)	280 (29%)
	No	4 (67%)	29 (59%)	692 (71%)
Histogenic subtype	SSM	1 (16.7%)	16 (33%)	597 (61%)
	Nodular	1 (16.7%)	8 (16%)	278 (29%)
	CGN	1 (16.7%)	0 (0%)	0 (0%)
	Other	3 (50%)	25 (51%)	97 (10%)
Mitotic rate	1 per 5 hpf	1 (16.7%)	7 (14%)	412 (42%)
	2-4 per 5 hpf	1 (16.7%)	9 (19%)	288 (30%)
	>5 per 5 hpf	2 (33%)	6 (12%)	272 (28%)
	Unknown	2 (33%)	27 (55%)	0 (0%)
Vascular invasion	Absent	4 (67%)	31 (63%)	896 (92%)
	Lymphatic	0 (0%)	1 (2%)	36 (4%)
	Blood vessel	0 (0%)	1 (2%)	16 (2%)
	Unknown	2 (33%)	16 (33)%	24 (2%)
Type of cell	spindle	0 (0%)	4 (8%)	139 (14%)
	epithelioid	2 (33%)	15 (31%)	787 (81%)
	Unknown /other	4 (67%)	30 (61%)	46 (5%)

mm: millimeter, SSM: superficial spreading melanoma, hpf: high power field CGN: Congenital giant naevus, i.s.: in situ

Table 2*: Histopathological data*

group. These models were then directly applied to the juvenile group in order to asses the impact and the weight of the explanatory variables on the subsequent dependent variables between both groups. To compare the two groups in Breslow thickness the Mann Whitney U test was used. Disease free survival was defined as the time between the date of diagnosis and the date of first recurrence. The follow up time was defined as the time between the date of diagnosis and the date of disease-specific death or last visit to the outpatient clinic. The Kaplan-Meier and Cox regression analysis tests were performed in order to calculate the 10-year DFS and 10-year overall survival curves with the date of diagnosis, first recurrence or death acting as end-points. To compare the survival distributions between the juvenile and adult groups a log rank test was used. A *p*-value less than 0.05 was considered to be significant. The software package SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL) was used for statistical analysis.

❑ Results

Clinical and Histopathological data

Children (under 12 years old)

Six children under the age of twelve years were identified to have a cutaneous melanoma (1 patient had an in situ melanoma, 1 Stage IA, 3 Stage IIB and 1 Stage IIIC). The median follow time was 65 (5 - 188) months. Of the six melanomas, two were located on the extremities (33.3%), two on the trunk (thorax and back, 33.3%) and two in the head and neck area (33.3%) (Table 1). The median Breslow thickness was 3.9 (range in situ – 5.0) mm (Table 2). None of the children were identified to have a dysplastic naevus syndrome or xeroderma pigmentosum. Of the six patients three developed local failure (50%) within 33 months after diagnosis (median time 9 (range 2-33) months). One patient developed distant metastases (16.7%). This patient had a congenital giant naevus that turned into a melanoma with a tumor thickness of 5.0 mm. This patient developed distant cutaneous and liver metastases within 3 months of diagnosis and died 2 months later. Of the other five patients, 2 are alive with disease (9 and 34 months) and three are disease free

(95, 130, and 188 months). The number of patients under the age of twelve was not sufficient for statistical analysis and therefore not considered.

Juvenile (between 12 – 19 years old)

Forty nine patients between the ages of 12 and 19 years old with a median age of 17 (range 12 – 19) years were identified to have a cutaneous melanoma (3 in situ, 11-IA, 5–IB, 9–IIA, 3–IIB, 4–IIC, 13–IIIB, and 1–IV). There were 26 males (53%) and 23 females (47%) (*p=ns* compared to the adult group, Mann Whitney U). The median follow up time was 92 (4 - 366) months. Compared to the adult control group, there were significantly more patients who presented with a locally advanced stage IIIB (26.5%) in the juvenile group than in the adult group (11.3%) (*p*<0.01, chi square test). One patient was previously diagnosed with an acute lymphatic leukemia which was treated successfully with chemotherapy. Two patients were diagnosed by the dermatologists with having a dysplastic naevus syndrome. No patients had xeroderma pigmentosum. In this group, 31 patients (63%) had extremity melanomas with 27 patients originating from the lower extremities (55%). Fourteen patients had trunk (thorax, abdomen and back) melanomas (29%) and 4 patients had head and neck melanomas (8%). There was no difference in tumor location between the juvenile and adult control group (p=0.538, chi square test). In the juvenile group the median Breslow thickness was 1.6 mm compared to 2.0 mm in the adult control group (*ns*, *p*=0.075 Mann Whitney U test). All histopathological data are summarized in Table 2.

Regression analyses for disease free survival and overall survival

Logistic and linear regression models were developed using DFS and OS as dependent variables and Breslow thickness, 2002 AJCC staging, age, gender, tumor location, histogenic subtype, mitotic rate, ulceration and vascular invasion as the independent variables. The models were set up for the control group as an exploratory regression analyses, with Conditional Forward Stepwise inclusion (OS) and Conditional Backward exclusion (DFS) of explanatory variables. Table 3 and 4 shows in condensed form the comparison of the model between the control and the juvenile group. In the DFS model, increasing Breslow thickness, increasing age, male gender, tumor location and the presence of ulceration were calculated to be significant predictors for disease free survival in the adult control group. None

Group	Variable	ß	Standard error ß	p-value	Expected ß	95% Confidence Interval for expected ß	
						Lower	Upper
Control	AJCC	0.385	0.044	<0.001	1.469	1.346	1.603
	Age	0.016	0.007	0.023	1.016	1.002	1.030
	Constant	-4.48	0.440	<0.001	0.011		
Juvenile	AJCC	0.617	0.245	0.012	1.854	1.148	2.994
	Constant	-4.86	1.545	0.002	0.008		

Table 3*: Logistic regression model for survival*

of these variables reappeared in the juvenile group as significant predictors for disease free survival.In the juvenile group, only the variable histogenic subtype showed more or less a significant explanatory power (p=0.056). The explained variance in both models did not differ; the adult control group model explained 11% variance while the model for the juvenile group explained 8% of the variance (R-square).

In the survival model, the explained variance in both control and juvenile models were very different. In the adult control group, 18% of variance was explained by age and AJCC stage. In the juvenile group, 34% of the variation was explained by the AJCC stage, and age was a non-significant contributor to the explanation of survival (Nagelkerke R-square).

10-year disease free and overall survival

There was no difference between the two groups regarding the time to develop local recurrence. For the Stage I/II melanomas the 10-year DFS was calculated to be 64% for the juvenile patients compared to 71% for the adult patients (*p*=0.236). The 10-year OS was respectively 79% and 83% (*p*=0.198). Juvenile patients with Stage III disease had a 10-year DFS of 45% compared to 40% for the Stage III adult patients (*p*=0.778). The OS for the Stage III patients was 50% for both groups (p=0.464) (Figs. 1-3).

Group	Variable	ß	Standard error ß	p-value
Control	Breslow	-1.776	0.656	0.007
	Age	-0.359	0.097	<0.001
	Gender	12.073	3.169	<0.001
	Location	-8.156	2.097	<0.001
	Ulceration	7.519	3.804	0.048
	Constant	83.507	-14.079	<0.001
Juvenile	Histogenic subtype	-8.276	4.220	0.056
	Constant	93.977	13.360	<0.001

Table 4: *Linear regression model for disease free interval*

❑ **Discussion**

Melanoma in childhood and during the adolescent years is rare. Approximately 1 to 4% of all new melanoma cases occur in patients under the age of 20 years and only 0.3% among those younger than 14 years.[4-6] Because of the low incidence, a correct diagnosis is difficult to make by the clinician. The degree of difficulty has increased since Sophie Spitz published her paper about melanomas in childhood in 1948.[8] Although there were large similarities in histological features, she noticed that many melanomas in children were rarely malignant in behavior compared to adults. Due to its benign clinical behavior the term Spitz naevus was introduced as a separate entity. Usually they are under 1 cm in diameter and may resemble verrucae or small hemangiomas. Melanomas in children tend to be larger and quite strikingly clinically. Histologically, Spitz naevi are characterized by large spindle or epitheloid cells with sharp cytoplasmic borders. Architectural features supporting the diagnosis of Spitz naevi include symmetry, associated epidermal hyperplasia, multinucleated giant cells, and a wedge shaped silhouette. In contrast, a lack of maturation, deep mitosis, marked nuclear pleiomorphism; hyperchromasia and an expansive architecture of the dermis are suggestive

features of melanoma.[13,14] Misdiagnosis is common and some melanomas are diagnosed when the tumor has already metastasized. Therefore all histological slides of all resected suspicious naevi in childhood should be reviewed by an experienced pathologist to minimize the misdiagnoses.

In our study of patients with childhood and juvenile melanoma, 29 patients were male and 26 were female. This slight male preponderance in sex distribution is not in accordance with the literature where a female preponderance has been reported.[15] This difference was however not significant. Compared to our adult group there were also more males than females but this difference was also not significant. Risk factors in the development of melanoma are; large congenital naevus (>2cm) which occurs in 1/20000 births and garment naevus (>20cm) occurring in 1/500000 births. Malignant transformation is estimated to be at about 5%.[16,17] The risk of melanoma arising in a large congenital naevus during the first year of life is estimated at 8.6 per 10,000.[18] Therefore prophylactic excision is recommended for large congenital naevi. In garment naevi, a lifelong clinical
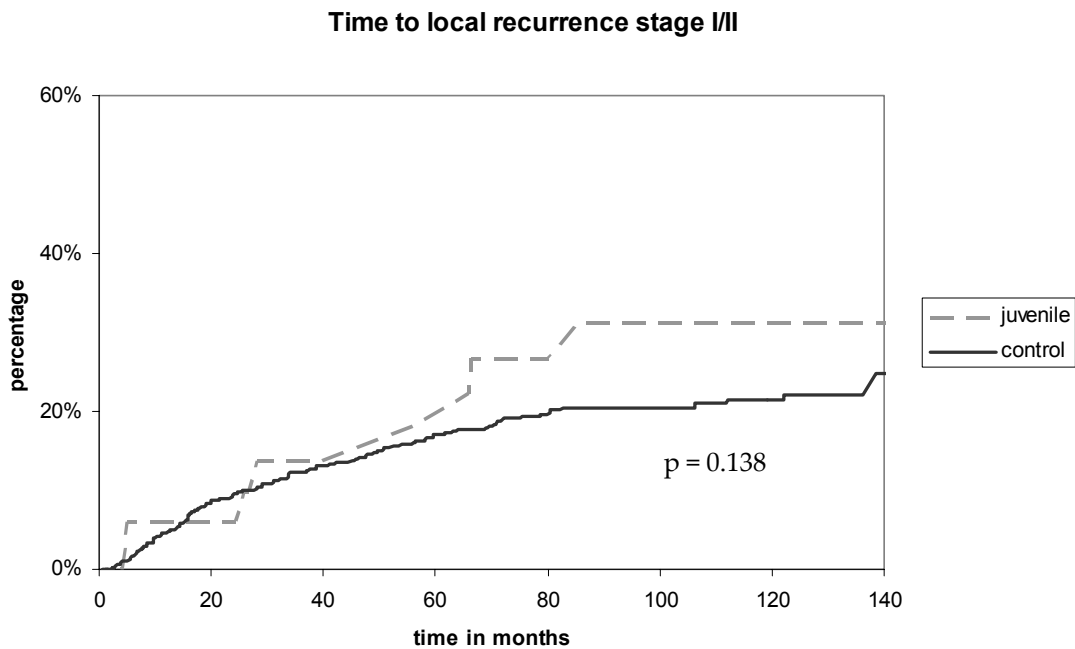
Time to local recurrence stage I/II

Fig 1. Time to local recurrence of the stage I/II juvenile and control group

114

surveillance is warranted. For small and medium sized congenital naevi (<2cm), treatment recommendations remain controversial.[19] If removal is desired, it is reasonable to wait until after puberty, when the child is better able to tolerate the procedure, because the risk of malignancy in these lesions prior to this period is exceedingly small.[4] Xeroderma Pigmentosum is an inherited disorder characterized by photosensitivity and defective cellular repair of DNA damaged by UV radiation. About 5% of these patients will develop melanoma at a median age of 19 years. Protective clothing and the use of sunscreen is recommended, and close monitoring of these patients is necessary at frequent intervals. A melanoma can develop in patients with dysplastic naevus syndrome as early as 10 years old, but usually they occur around the age of 15 due to an increase in sunlight exposure. The appearance of a new area of black pigmentation in a dysplastic naevus may be the best indicator of early malignant change.
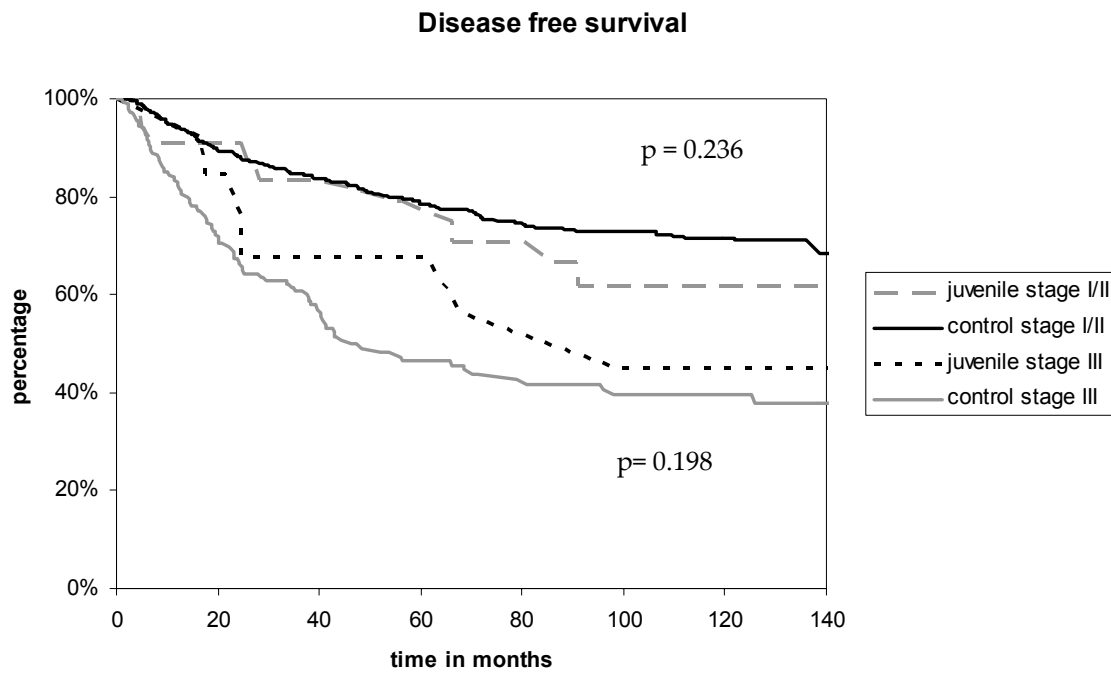
Disease free survival

Fig 2. DFS juvenile vs. control group
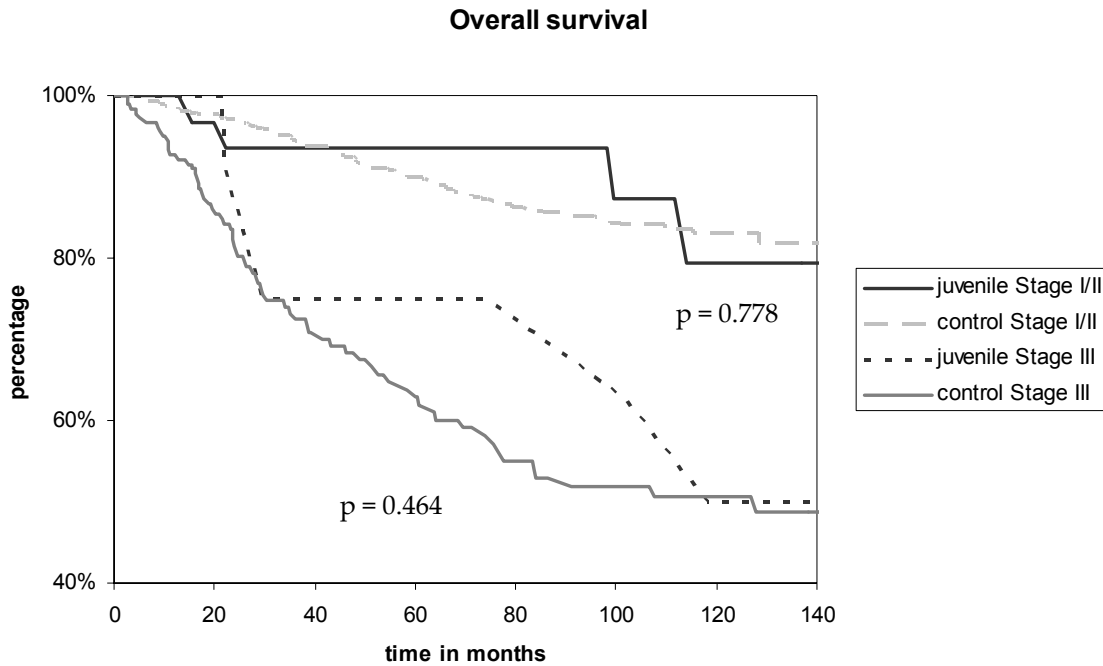
Overall survival

Fig 3. *Overall survival juvenile vs. control group*

Historically it is believed that chronic sunlight exposure causes the malignant transformation but recently researchers at the National Cancer Institute reported that even a single exposure of strong sunlight during childhood can lead to melanoma.[20]

Previous reports suggested that the thickness of the melanoma and the level invasion are the most important predictors of long-term survival in young patients.[21,22] In our series of juvenile melanomas,   confirm these findings. Regarding DFS and OS, we demonstrated that Breslow thickness, male gender, tumor location (trunk), ulceration, increasing age and stage at diagnosis were independent prog   es for the adult group. However, in the juvenile group only the sta   is was calculated to be a prognostic factor. This is an interesting finding considering that in our series there was a significant difference in the amount of patients (27%) that presented with a locally advanced stage IIIB compared to the adult control group (11%). A possible explanation may

116

be a delay in treatment by patient, parent or physician or even a histopathological misdiagnosis. Usually there is a delay in clinical diagnosis and occasionally a failure of the patient and/or parent to seek medical help.[23,24] It is therefore imperative for physicians to refer these patients to a specialized center in case of any doubt regarding a naevus in the young for an excisional biopsy. Despite the amount of patients diagnosed at an advanced stage, the 10-year survival rate did not significantly differ between the adult and juvenile groups. We report a 10 year DFS of 64% for Stage I/II juvenile patients and 45% for the Stage III juvenile patients. A 79% 10-year OS was calculated for the Stage I/II juvenile group and 50% for Stage III group. Tate et al. reported comparable rates of DFS in children to those in adults with Stage I/II disease: approximately 77% at 5 years.[25] This is accordant to our results at 5 years (Fig.2). In a recent population-based study in Europe the 5-year overall survival for childhood (0-14 yrs) melanoma was 79%.[5] Data available from the USA Surveillance, Epidemiology and End Results (SEER) shows, for the age group of 10-14 years, a 5-year overall survival of 86%.[26] These studies are not entirely comparable because of the age groups studied compared to our study (10-14yrs vs.12-19yrs).

In conclusion, there seems to be no difference in the 10 year DFS and OS between the two groups even though juvenile patients presented more often with a locally advanced stage. Also the known prognostic factors do not apply for the juvenile patients, except for the stage at diagnosis. The factors that affect the DFS and OS are not yet known and further research is necessary to determine which factors are responsible for the course of this disease in juvenile patients. Depending upon the results of the Morton trial it will be clear whether there is a role for sentinel lymph node biopsy in these young patients. It still remains imperative that clinicians recognize the occurrence of this disease in younger patients and that it can follow an aggressive course. Prompt excision of suspected pigmented naevi is warranted and referral to a specialized center is justified.

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Chapter VII

❑   Brain metastases in cutaneous head and neck melanoma

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❑ Introduction

Cutaneous melanomas are tumors with an unpredictable biological behavior. The worldwide incidence of melanoma is increasing dramatically and despite significant advances in prevention, early detection and treatment, the mortality rate continues to increase steadily.[1-3] Currently the incidence rate and mortality rate in the Netherlands is respectively 12.6 and 3.1 per 100,000 people, with 2100 new cases of melanoma being diagnosed in 1998.[4] Cutaneous melanoma of the head and neck region comprise 10-20% of all melanoma.[5-7] Patients with head and neck melanoma have a worse prognosis than those with extremity or axial lesions, with an overall 5-year survival of approximately 65%.[8] However, if local recurrence develops the 5-year survival drops to 36%.[9]

Melanoma represents the third most common cause for the development of brain metastases, after primary tumors of the lung and breast.[10,11] Schouten et al. reported an incidence of 7.4% from a cohort of melanoma patients of all stages and primary locations.[11] The question has been risen as to if there is a difference in incidence in brain metastases between head and neck cutaneous melanoma and extremity/truncal melanoma which could explain the difference in survival between these two groups. As a result of the increasing number of melanoma cases, the incidence of brain metastases is also likely to increase in the coming decade. It has therefore become important to investigate and identify the risk factors associated with this devastating complication so that patients at highest risk can be monitored more closely and possibly treated earlier to improve the survival and quality of life.

❑ Patients and Methods

Between 1965 and 2000, a total of 2567 patients were diagnosed and treated for a cutaneous melanoma at the Groningen University Medical Center. The population consisted of 991 male patients (39%) and 1576 female patients (61%). During this period, 324 (13%) patients of which 152 female (47%) and 172 male (53%), were treated for a cutaneous melanoma of the head and neck. The median age was 57.5 (range 4.3-93.5) years with a median Breslow thickness of 2.1 (range 0.1-13.0) mm.

The melanomas were staged according to the 2002 American Joint Committee on Cancer (AJCC) staging system.[12] The clinical and pathological data was retrieved from the Groningen melanoma database. Patients who were classified as having head and neck cutaneous melanomas and brain metastases were identified as the study group. The clinical data retrieved included gender, age at diagnosis, site of the primary tumor, and stage at diagnosis. The histopathological characteristics recorded included the histologic subtype of melanoma (superficial spreading, nodular or other/unknown), Breslow's depth of invasion (in millimeters), tumor ulceration, the presence or absence of vascular and/or lymphatic invasion and mitotic rate per 5 high power fields (per mm^2). All histological slides were reviewed at our institution.

Treatment

All patients were treated according to the guidelines of the Dutch Melanoma Working Party.[13] Most patients, who were referred to the Groningen University Medical Center, underwent excision of their primary lesions elsewhere. The patients were referred for further treatment consisting mostly of wide excision only, in certain cases in combination with an elective or therapeutic lymph node dissection. A sentinel lymph node biopsy was performed since 1995.

Follow up

All patients were regularly followed in the outpatient clinic for regional and/or distant failures. The follow up included history, physical examination and yearly

	Total group Head and Neck Patients	Head and Neck Patients with Brain metastases	Extremity / Axial Matched control group
Total Patients	324	26	26
Age median	58	46	54
(range)	(4 – 94) years	(16 – 79) years	(24 – 76) years
Male	172 (53%)	19 (73%)	11 (42%)
Female	152 (47%)	7 (27%)	15 (58%)

Table 1: *Clinical data*

chest x-rays. It did not include lab controls or regularly scheduled CT scans of the brain. Regional failure was defined by the histological or cytological confirmation of tumor cells at the site of the primary tumor and/or along its lymphatic drainage pathway to the first lymph node basin. Distant failure was defined by radiological detection of suspected lesions by means of CT (lungs, brain), ultrasound (liver) and bone scintigraphy. Before the introduction of these diagnostic tools, distant metastases were diagnosed clinically. Appropriate treatment of recurrences was performed depending of the type and site of recurrence. No patient received any kind of adjuvant systemic treatment. Patients with brain metastases were treated either with surgery, radiation therapy, steroids, chemotherapy or a combination of these.

Incidence of brain metastases and matched controls

The incidence of brain metastases for the extremity/truncal group was calculated by using a subgroup of patients of the database. This subgroup consisted out of 518 males and 861 female patients and which presented which Stage I-III disease.

A matched control analysis between the head and neck patients who did develop brain metastases and a match control group from the extremity/truncal melanoma was performed to isolate risk factors. To verify these results, we isolated these factors and performed the same analysis on our complete database of head and neck patients that did not develop brain metastases (298 pts).

Statistics

The software package SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL) was used for statistical analysis. To calculate the difference in incidence of brain metastases the exact confidence interval construction was used for the test of binomial populations. To compare the study group and the matched control group, cross tables and the chi-square test were used. The logistic regression method (multivariate) was used to verify our results with the complete database of cutaneous head and neck melanoma.

		Total group Head and Neck Patients	Head and Neck Patients with Brain metastases	Extremity / Axial Matched control group
Breslow thickness (mm)	0 – 0.75	123 (38%)	3 (12%)	0 (0%)
	0.76 – 1.50	51 (16%)	3 (12%)	6 (23%)
	1.51 – 4.00	96 (29%)	8 (30%)	15(58%)
	> 4.01	54 (17%)	12 (46%)	5 (19%)
	Median (range)	1.3 (0.1 – 13.0)	3.3 (0.7 – 12)	2.9 (0.9 – 9.8)
Ulceration	Yes	64 (20%)	10 (38%)	9 (35%)
	No	166 (51%)	13 (50%)	17 (65%)
	Unknown	94 (29%)	3 (12%)	0 (0%)
Histogenesis	SSM	80 (25%)	10 (38%)	19 (73%)
	Nodular	54 (17%)	8 (31%)	6 (23%)
	Other	190 (58%)	8 (31%)	1 (4%)
Mitotic rate	1 per 5 hpf	78 (24%)	3 (12%)	7 (27%)
	2-4 per 5 hpf	52 (16%)	7 (27%)	13 (50%)
	>5 per 5 hpf	50 (15%)	11 (42%)	6 (23%)
	Unknown	144 (45%)	5 (19%)	0 (0%)
Vascular invasion	Absent	205 (64%)	19 (73%)	25 (96%)
	Lymphatic	8 (2%)	1 (3%)	0 (0%)
	Blood vessel	7 (2%)	3 (12%)	1 (4%)
	Unknown	104 (32%)	3 (12)%	0 (0%)

mm: millimeter, SSM: superficial spreading melanoma, hpf: high power field

Table 2: Histopathological data

Disease free survival (DFS) was defined as the time between the date of diagnosis of the primary tumor and the date of diagnosis of brain metastases. The follow up time was defined as the time between the date of diagnosis and the date of disease-specific death or last visit to the outpatient clinic. The Kaplan-Meier and Cox

regression analysis tests were performed in order calculate the DFS and the overall survival curves. A *p*-value less than 0.05 was considered to be significant.

❑ Results

Clinical and histopathological data

Twenty-six of the 324 patients with head and neck melanoma (8%) were found to develop brain metastases after a median follow up period of 24 (range 4-75) months. The median age of the study group was 46 (range 16-79) years. The median total follow up time for the patients who developed brain metastases was 2.3 yrs (range 0.6 – 11.5 yrs). Of the 26 patients, 4 were Stage I, 10 Stage II, and 12 Stage III (specifically: 2-IA, 2-IB, 3-IIA, 4-IIB, 3-IIC, 3-IIIA, 8-IIIB, and 1-IIIC). The median Breslow thickness was 3.3 (range 0.7-12) mm. See Table 1 and 2 for the complete clinical and histopathological data of the study group, matched control group and total group of head and neck patients. In comparison, the incidence of extremity/truncal melanoma with brain metastases was 5.2% (CI 0.058-0.108, P<0.05). Ten of the twelve Stage III patients underwent a therapeutic lymph node dissection, 1 Stage IIB and 1 Stage IIC patient had an elective lymph node dissection and 1 Stage IIB patient underwent a sentinel lymph node biopsy. The treatment of brain metastases consisted out of: steroids and radiation in 9 patients (35%) (total 30 Gy in 10 fractions of 3 Gy), chemotherapy in 2 patients (8%) (1 patient a combination of carboplatin and cytarabine, 1 patient a combination of imidazole carboxamide (DTIC), cisplatin and vinblastin), while in 2 patients (8%) with a single brain metastasis a metastasectomy was performed with adjuvant radiotherapy. Steroids alone (dexamethasone) were prescribed for the remaining 13 patients.

Time to brain metastases (DFS) and overall survival

For the Stage I/II head and neck patients which developed a brain metastasis, the median time to brain metastasis was 24 (range 4 – 75) months. For the Stage III patients the median time was 20 (range 4 – 75) months (Figure 1). The DFS between Stage I/II and Stage III was not significantly different. The median survival for all 26 patients was 2.4 (range 0.2-64.3) months, and the one-year survival was 15%

126

after developing brain metastases (Figure 2). The primary site of distant failure in the Stage I/II group was 71% (10/14) in the brain and 29% (4/14) in the lungs. For the Stage III patients the percentages were 59% (7/12) brain, 33% (4/12) lungs and 8% (1/12) bone. Thirteen of the 26 patients (50%) developed multifocal brain metastases, 3 patients (12%) developed a single metastasis and in the remaining 10 patients (38%) the medical records did not mention details with respect to the extent of the brain metastases.

Prognostic factors

From our cross-tables analysis (Table 3) it shows that the variables Breslow thickness, gender and mitotic rate have discriminating power between the study group and the match control group. The head and neck group with brain

	HN + vs. ET match control		HN + vs. HN -	
	Chi- square	p- value	Chi- square	p- value
Breslow thickness **(>4 mm)**	9.01	0.029	16.5	<0.001
Gender **(Male)**	5.04	0.025	4.45	0.035
Mitotic rate **(>5 per 5 hpf)**	9.87	0.02	20.6	<0.001

HN + : Head and neck patients with brain metastases; HN - : Head and neck patients without brain metastases; ET: extremity / truncal patients; mm: millimeter; hpf: high power field

Table 3: *Chi-square analysis between the head and neck group with brain metastases and the matched control group; and between the head and neck group with and without brain metastases.*
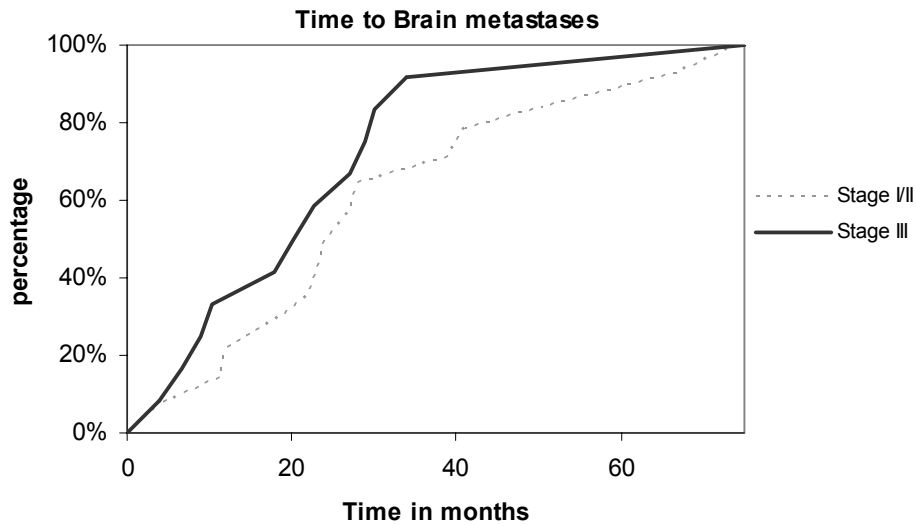
Fig. 1: *Time to Brain metastases*

metastases revealed a higher score on Breslow thickness greater than 4 mm, there are more males and the head and neck-group has a significant higher mitotic rate (>5 per 5 hpf).These results were checked in a broader spectrum by performing the same cross tables analysis on our complete database of 298 head and neck patients. This analysis confirmed Breslow thickness greater than 4 mm, male gender and mitotic rate as prognostic factors for developing brain metastases (Table 3). Subsequently the logistic regression analysis with these variables revealed that besides the variables Breslow, mitosis and gender, younger age ($p < 0.05$) entered as an additive variable (Table 4).

❑ **Discussion**

The incidence of brain metastases from cutaneous head and neck melanoma has been hypothesized to be higher than trunk or extremity melanomas. The incidence of brain metastases from head and neck melanoma was in this series 8% vs. 5.2% from the extremity/truncal melanoma (Stage I-III). To our knowledge, only two other clinical series have previously reported data on this subject. These authors
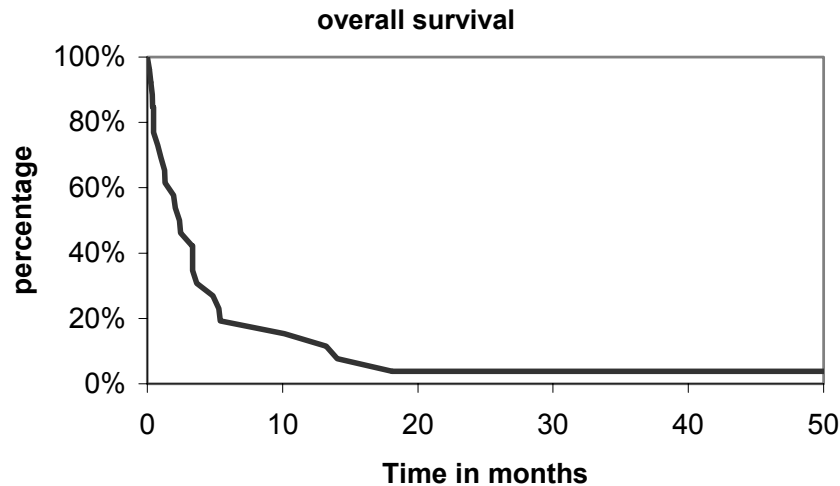
128

overall survival

Fig. 2: Overall survival after developing brain metastases

reported an incidence of 11% metastases to the central nervous system from all head and neck melanoma cases (Stage I-IV).[10,14]Furthermore, they claim that 19.6% of all metastases to the central nervous system from melanoma cases originate from the head and neck region. Van de Vrie reported that more than 40% of the patients with loco-regionally metastasized melanoma (Stage III) of the head and neck region developed cerebral metastases. They therefore recommend a neurological examination and a CT scan of the brain prior to a lymph node dissection.[15] In our series, from the 95 head and neck melanoma patients who presented as Stage III or became Stage III from Stage I, 19 (20%) patients developed brain metastases. An interesting observation is that in most of the patients that did develop brain metastases, this was also the primary site of distant failure suggesting a clear affinity for this pathway of dissemination. The most common symptoms of brain metastases were headaches, unsteadiness, nausea, cranial nerve palsy and disorientation due to increased intracranial pressure or local pressure on the affected structure. The median interval time from excision of a histologically confirmed primary lesion to a diagnosis of brain metastases was 24 months in the Stage I/II patients and 20 months in the Stage III patients with the longest interval being 75 months. Retsas et al. reported similar interval times of 29.6 months to

brain metastases from all cutaneous melanoma.[16] Our results show a median overall survival of 2.4 months and a 1-year survival of 15% after developing brain metastases which is comparable with a previous report published by Sampson et al.[14] The most significant factor influencing survival of patients with brain metastases is the number of other involved organs with metastatic disease. Consistently, Stage IV patients with metastases limited to the brain have had a significantly better outcome.[14,17-19]

As can be seen from our results, a Breslow thickness greater than 4 mm and a higher mitotic rate (>5 per 5 hpf) increases the risk of the patient falling into the risk group of developing brain metastases. It is well accepted in the literature, that increasing Breslow thickness and increasing mitotic rate, have a negative effect on disease free and overall survival.[12,20,21] It is therefore that Breslow thickness has been included as an independent prognostic factor in the new American Joint Committee on Cancer staging system. However the other main prognostic factor, tumor ulceration, was not found to be a determining factor in our study in developing brain metastases. Other known prognostic factors such as nodular tumor type and vascular invasion were also not determining factors of developing

Variable	Regression coefficient (ß)	P value
Breslow thickness	0.59	0.012
Mitotic rate	0.459	0.020
Gender	-0.837	0.084
Age	-0.24	<0.05

R^2 *(explained variance) = 0.21 (p<0.01)*

Table 4: *Variables associated with brain metastases from HN melanoma (Multivariate regression analysis)*

130

brain metastases. This last one is surprising considering that the primary site of distant failure in all stages was the brain, which by definition is a hematogenous spread. Our results also showed that is not so much being male that has an added risk on the development of brain metastases, but that being female has a protective effect against the development of brain metastases. Similarly being older also had a protective effect. Based on the current findings, clinicians should be aware of the increased risk of male patients and younger patients of developing brain metastases compared to the female and older patient.

It has been previously reported that patients who develop single brain metastasis have a better overall survival than patients with multiple brain metastases. Two prospective randomized clinical trials by Patchell and Vecht, in which patients with single metastases and stable extra cranial disease were treated either with surgery followed by radiotherapy or radiotherapy alone, showed a significant lower recurrence rate and longer overall survival in the surgical group.[22,23] These trials however included mostly primarily nonsmall-cell lung cancer as the primary tumor and involved only nine patients with metastatic melanoma. The question still remains if this data is applicable for melanoma metastasis alone. The current literature does seem to suggest that surgical resection for single or limited brain metastasis, which could be easily resected prolongs the disease free interval and overall survival when extra cranial tumor is absent or stable during the last three months.[24,25] From our 26 patients which developed brain metastases, the only patient surviving had a single metastasis which was surgically resected followed by adjuvant radiotherapy. In recent years, gamma knife stereotactic radiosurgery has gained considerable momentum as a major alternative in the management of intra-cranial metastatic melanoma in selected cases.[19,26,27] And whole brain irradiation alone has proven to be a useful therapy in the palliative setting.[28] The role of chemotherapy for brain metastases is limited and still controversial. Melanoma is traditionally not a very chemosensitive disease. Adding to that, limited life expectancy and presence of the blood-brain barrier have been considered contraindications for relatively aggressive therapies such as chemotherapy. There is therefore no standard chemotherapeutic treatment for Stage IV disease. In the Netherlands, Stage IV patients are usually treated in clinical trials if possible. If not possible then the patient can be treated with DTIC.[29] Newer drugs such as topotecan and temozolomide seem to be particular promising

(alone or in combination with other drugs) in the treatment of brain metastases from cutaneous melanoma.[30]

In conclusion, brain metastasis develops in 8% of all head and neck melanoma cases compared to 5.2% from extremity/truncal melanoma. Breslow thickness, mitotic rate, male gender and younger age have been isolated to be risk factors in developing brain metastases in head and neck melanomas. The reason why the head and neck melanomas with these characteristics primarily disseminate to the brain so frequently is still unclear. The head and neck region is of course more exposed to sunlight and has a rich amount of lymphatic vessels. But these factors are not determining in the development of a hematogenous spread such as to the brain. Further research is necessary to unveil this specific pathway of dissemination. The current literature suggests that surgical resection followed by radiotherapy for limited metastasis may prolong survival and the quality of life.

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134

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Chapter VIII

❑   Summary, conclusions and future perspectives

□   **Part A   Hyperthermic Isolated limb perfusion**

The theory behind the delivery of isolated regional chemotherapy is that a high drug uptake by the tumor may be achieved with higher doses of chemotherapeutic agents without increasing the systemic toxicity. The technique of isolated limb perfusion, a regional cancer treatment that allows the direct infusion of high doses of chemotherapeutic agents in the arterial supply of a tumor-bearing area of a limb, came available with the development of an extra-corporeal circuit for cardiac surgery (**chapter 2**). In the mid fifties the surgical-oncologists Creech and Krementz of the Tulane University in New Orleans developed the technique of the isolated limb perfusion. The first isolated limb perfusion was performed at the Groningen University Medial Center in 1964. In the sixties Cavaliere reported on selective heat sensitivity of tumor cells and added hyperthermia within the regional perfusion technique. Key point in isolated limb perfusion is that the dose of chemotherapeutic agents used, can be 15-20 fold the maximum systemic tolerated dose, since vital organs are isolated from the perfusion circuit. In the beginning isolated limb perfusion was used as a therapeutic treatment for locally advanced melanoma, recurrences and/or intransit metastases. Later on adjuvant limb perfusions were introduced in the treatment of melanoma of the limb, but an EORTC randomized multi center trial showed no real benefit of hyperthermic isolated limb perfusion as an adjuvant treatment modality for patients with high-risk stage I melanoma (> 1.5 mm Breslow thickness). On the other hand isolated regional perfusion with melphalan is a well accepted treatment for intransit metastases and/or recurrences in patients who are no longer candidates for local treatment, e.g. surgery, cryotherapy or laser treatment. Other chemotherapeutic agents have been used in melanoma perfusions, showing lower subjective response rates and often higher toxicity. A variety of other antineoplastic agents besides melphalan have been used during the last forty years in hyperthermic isolated limb perfusion such as darcarbazide (DTIC), actinomycin-D, thiotepa, mitomycine-C, doxorubicin and more recently cisplatin. The majority of the cytostatic agents were or ineffective, or the duration of response quite limited, or when a drug showed effectiveness the local toxicity hampered the further application. Doxorubicine showed effectiveness but disturbed the arterial vascularity of the

limb, while cisplatin was too neurotoxic. In the late nineties Lejeune introduced TNFα in the perfusion setting together with melphalan in the treatment of locally advanced melanoma and sarcoma of the limb. In a randomized trial between melphalan and TNFα with melphalan for locally advanced melanoma, no real benefit was seen by including TNFα, compared to melphalan alone with a median survival of 2.5 years. Patients with low tumor burden or small tumors showed equivalent results (TNFα and melphalan versus melphalan), while TNFα and melphalan showed higher response rates in bulky melanoma. Therefore melphalan is the drug to be used in melanoma perfusion and combined with TNFα when bulky disease or recurrent disease after previous melphalan perfusion is the indication for perfusion.

In **chapter 3** a derivative of cisplatin, carboplatin was used in a feasibility study with regard to effectiveness and toxicity in the management of locally recurrent and/or intransit metastases of melanoma or locally advanced soft tissue sarcoma. Three patients, two with locally advanced melanoma and one with a low grade liposarcoma of the lower extremity, were treated with ILP under mild hyperthermia (39 - 40 C) with 125 mg carboplatin/L perfused limb volume. No systemic toxicity was observed. Local toxicity consisted of postperfusion edema present in all 3 patients which resolved within 2 weeks. Clinically a persistent local neuropathy was observed in all 3 patients, 2 of which were confirmed by electromyogram and nerve conduction study. The severe motor-sensory neuropathy was located mainly in the peroneal and sural nerves of the perfused limbs. Pharmacokinetic parameters of the carboplatin showed a higher concentration of carboplatin in the skin compared to the muscle. The two melanoma patients showed a complete response but developed local recurrences within 12 months after popliteal and femoral perfusion. The third patient underwent a delayed excision of the sarcoma 8 weeks after perfusion which revealed 50% viable tumor. One of the melanoma patients and the sarcoma patient died from lung metastases respectively 56 and 31 months postperfusion treatment. The other melanoma patient is alive 95+ months post perfusion treatment. We concluded that the local neurotoxicity observed did not warrant further research of carboplatin in ILP.

With the introduction of recombinant tumor necrosis factor α (TNFα) in the early nineties, the effectiveness of the ILP was increased for melanoma and especially for STS patients. However, leakage of TNFα into the systemic circulation gives rise to more severe side effects than melphalan alone. A 1% leakage may already result in hypotension of the patient and a 10% leakage of TNFα can cause a potentially fatal septic shock like syndrome. However, strict isolation of the limb is not always achievable due to anatomical variations and/or technical reasons. Minimal systemic leakage can therefore not always be prevented, and makes continuous leakage monitoring during the perfusion procedure of utmost importance. In the period 1977 – 1991, 331 perfusions were performed at this institution with a median leakage of 8.0% (range 0 – 30%) using melphalan. In **chapter 4** we calculated our leakage of perfusate into the systemic circulation using radioactive tracers in the patients who were perfused after 1991. Coincidentally since 1991, the very toxic TNFα has been added to the treatment regimen for ILP. The aim of the study was to assess the changes in leakage rate between the periods of 1977-1991 and 1991-2000 and to determine the factors responsible for these changes. One hundred and nineteen patients underwent ILP mainly for locally recurrent melanoma or locally advanced soft tissue sarcoma. ILP was performed with melphalan (33%) or in combination with TNFα (65%). There were 67 iliacal (56%), 12 femoral (10%), 25 popliteal (21%) and 15 axillary (13%) perfusions performed. Leakage to the systemic circulation was monitored continuously with the help of ^{131}I-albumin and a stationary scintillation detector placed above the heart. The median maximum leakage was 2.7% (range 0-21%) which is significantly less than the previous period of 1977-1991 where leakage of 8% (0-30%) was previously reported (P<0.05). A statistical difference of the leakage was detected between the levels of perfusion were the iliacal and femoral levels showed more leakage than the axillary and popliteal levels (P<0.05). Furthermore when TNFα was used, there appeared to be significant less leakage than when melphalan was the sole drug (P<0.05). We concluded that nowadays leakage from isolated perfusions into the systemic circulation is further minimized compared to the days when melphalan was the sole drug used. Increased awareness for TNFα leakage, continuous external monitoring with ^{131}I-albumin as the main isotope, flow rate regulation in the perfusion circuit and regulation of the systemic blood pressure of the patient have all been major contributors to this improvement.

140

❑   **Part B**   **Uncommon aspects of melanoma**

Pregnancy

Melanoma is often diagnosed in young adults and a significant number of these patients are women in their reproductive phase of life. The real incidence of melanoma during pregnancy is unknown. It has been suggested that women who present with a melanoma during pregnancy have a worse prognosis due to a more aggressive behavior of the melanoma due to an influence of hormonal or reproductive factors. In **chapter 5** the aim of the study was to evaluate the long-term effect of pregnancy on the progression of the disease in Stage I/II melanoma. During the period 1965-2001, 46 pregnant women were treated for a Stage I/II melanoma. These patients were compared with an age-sex control (non-pregnant) group of 368 Stage I/II patients. The patients were staged according to the 2002 AJCC TNM melanoma staging system. The 10-year disease free survival (DFS) and 10-year overall survival (OS) were calculated using the logistic regression method. The median age for the pregnant patients was 30 (range 18-46) years and 36 (range 17-45) years for the non-pregnant patients. Median follow-up time was 109 (range 1-356) months. Pregnant patients presented more often with thicker melanomas (median 2.0 vs. 1.7 mm, *ns*). No difference in tumor location, histologic subtype, tumor ulceration and vascular invasion could be detected between the pregnant and non-pregnant group. There was no statistical difference in the 10-year DFS and the 10-year OS between the 2 groups. The 10-year DFS for the pregnant patient and non-pregnant group was respectively 88% vs. 86% for the Stage I patients, and 67% vs. 73% for the Stage II patients. The 10-year OS was respectively 94% vs. 90% for the Stage I patients and 82% vs. 81% for the Stage II patients. We conclude that pregnancy does not appear to adversely affect long-term survival in patients with clinically localized melanoma.

Childhood and Juvenile melanoma

Melanoma before puberty is rare, but the incidence increases steadily thereafter in each decade. The steepest trends of increasing incidence are in those aged over 65 and the lowest trends are in the younger age groups. Approximately 1 to 4% of all new melanoma cases occur in patients under the age of 20 years and only 0.3%

among those younger than 14 years. In **chapter 6** we analyzed the childhood and juvenile melanomas treated at our institution with a special focus on differences in clinical characteristics, prognostic factors, disease free and overall survival in comparison with those of adults. During the period 1965-2003, 55 patients under the age of 19 years (26 female (47%) and 29 male (53%)), were treated for a cutaneous melanoma. These patients were compared with an adult control group of 972 patients. The patients were staged according to the 2002 AJCC TNM melanoma staging system. Using logistic regression analyses, the 10-year disease free survival (DFS), 10-year overall survival (OS) and independent prognostic factors determining OS and DFS were calculated for the juvenile as for the adult group. Six patients were under the age of 12 and therefore not considered for statistical analysis. The median age for the juvenile patients was 17 (range 12 - 19) years and 49 (range 20 – 93) years for the adult control patients. Median follow-up time was 92 (range 4 - 366) months. The Breslow thickness was comparable in both groups (juvenile median 1.6 mm vs. adult 2.0 mm, p=0.075). Juvenile patients did present more often with locally advanced melanoma (p<0.01). With regard to the DFS and OS, increasing age, mitotic rate, ulceration, Breslow thickness, male gender and stage at diagnosis all were calculated to be independent prognostic factors for the adult group. Where as in the juvenile group only the stage at diagnosis was a significant factor. There was no statistical difference in the 10-year DFS and the 10-year OS between the 2 groups. The 10-year DFS for the juvenile patients and adult group was respectively 64% vs. 71% for the Stage I/II patients, and 45% vs. 40% for the Stage III patients. The 10-year OS was respectively 79% vs. 83% for the Stage I/II patients and 50% vs. 50% for the Stage III patients. We concluded that there is no difference in the 10 year DFS and OS between the two groups even though juvenile patients presented more often with a locally advanced stage. Also the known prognostic factors do not apply for the juvenile patients, except for the stage at diagnosis. These factors are not yet known and further research is necessary to determine which factors are responsible for the course of this disease in juvenile patients.

Brain metastases from Head and Neck melanoma

There is an increasing incidence of cutaneous melanoma (CM), and 20-25% of CM is located in the head and neck region. In **chapter 7** the incidence of brain

142

metastases, risk factors and outcome were analyzed for the melanomas originating in the head and neck region. During the period 1965-2000, 324 patients of which 152 female (47%) and 172 male (53%), were treated at the GUMC for a CM of the head and neck. The patients were staged according to the 2002 AJCC melanoma staging system. A matched control analysis was performed in order to identify the risk factors for the occurrence of brain metastases. The analysis was performed using cross tabulations, chi-square test and logistic regression method. Twenty six (8%) HN compared to 5.2% (CI 0.058 – 0.108, $P<0.05$) of the extremity/ truncal patients developed brain metastases. The 26 head and neck patients (4 Stage I , 10 Stage II, and 12 Stage III) had a median age of 46 (range 16-79) years and developed brain metastases after a median follow up of 24 (range 4-75) months. The median Breslow thickness was 3.3 (range 0.7-12) mm. The patients were treated either with steroids, surgery, radiation, chemotherapy or a combination of these. The median survival after developing brain metastases was 2.4 (range 0.2-64.3) months with a one-year overall survival of 15%. Risk factors identified for developing brain metastases from head and neck melanoma are younger age, male gender, Breslow thickness greater than 4 mm and mitotic rate.

In conclusion, head and neck CM tend to develop brain metastasis in 8% compared to 5.2% in extremity/ truncal melanoma and is associated with the above mentioned prognostic factors. The prognosis of these cases is extremely poor with the available therapies. Metastasectomy and adjuvant irradiation for a single brain metastasis may prolong survival in selected cases.

❑ **Conclusions**

- Hyperthermic Isolated limb perfusion with melphalan has evolved to be an accepted palliative treatment for intransit metastases and/or recurrences in patients who are no longer candidates for local surgical treatment.

- Hyperthermic Isolated limb perfusion with carboplatin has proven to be ineffective in treating locally advanced melanoma and is accompanied by a severe neurotoxicity to the perfused limb.

- Continuous external monitoring with ^{131}I-albumin as the main isotope is a reliable method for detecting leakage of the perfusate to the systemic circulation.

- Pregnancy does not adversely affect the long-term outcome in patients who are diagnosed with a primary Stage I or II melanoma.

- The long term outcome of children/juvenile melanoma is comparable with that of adults. However, besides the stage of the disease at presentation, the known prognostic factors do not seem to apply for the children/juvenile melanoma.

- There is an increase in brain metastases from cutaneous head and neck melanoma compared to extremity/truncal cutaneous melanoma.

❑ **Future Perspectives**

Although conventional ILP is an effective method for treating recurrent limb melanoma, it is a complex and invasive procedure. Patient selection is rigorous and many patients can therefore not be treated. Patients with systemic metastases as well as limb recurrences, or patients with medical co-morbidities were usually excluded from ILP therapy. Since 1994 the Sydney melanoma unit introduced a simplified form of ILP called isolated limb infusion (ILI). This new technique was in fact a low-flow ILP performed under hypoxic conditions via small caliber arterial and venous catheters which are inserted percutaneously via the contralateral groin. Because of its low-pressure system, a pneumatic tourniquet is sufficient to control the leakage of the drugs into the systemic circulation. It is however not possible to raise the limb temperature above 38.5° C. The ILI procedure has been shown to be of similar efficacy to ILP and can be used in the elderly and medically compromised patients. Furthermore, the procedure can be repeated and systemic metastases are not an exclusion criteria.

Cutaneous melanoma is presently still an aggressive disease with an unpredictable biological behavior. The only curative treatment consists of radical surgical therapy of the primary tumor including its metastatic deposits. In the forthcoming years the value of the sentinel lymph node biopsy will become clear which role it will occupy in the treatment of Stage I melanoma (MSLT I and II study). Also it will become clear if the whole-body CT or PET scan has any value in further (up)staging the clinically Stage III patients (OMSPECT study). For the treatment of Stage III and IV patients, the polyvalent melanoma vaccination is being researched for its effectiveness. The value of frequent and long term outpatient visits in the follow up will be investigated for its effectiveness (MELFON study). All of the above mentioned studies are supposed to improve the treatment of melanoma patients who are condemned with this devastating disease.

Chapter IX

❑   Samenvatting, conclusies en vooruitblik

❑　Hyperthermische Geïsoleerde Ledemaat Perfusie

Het doel van geïsoleerde, regionale toediening van hoge doses chemotherapie is een versterkte cytostaticum opname in de tumor, zonder systemische toxiciteit te genereren. De mogelijkheid van geïsoleerde ledemaat perfusie (ILP), een vorm van regionale kankerbehandeling door infusie van hoge doses chemotherapeutica in de arteriële aanvoer van een ledemaat, kwam beschikbaar met de ontwikkeling van de extra-corporele circulatie ten behoeve van de hartchirurgie (**hoofdstuk 2**). De techniek voor de geïsoleerde ledemaat perfusie werd midden jaren vijftig ontwikkeld door de chirurgisch oncologen Creech en Krementz van de Tulane Universiteit te New Orleans. In het Academisch Ziekenhuis Groningen werd de eerste ledemaat perfusie in 1964 met succes uitgevoerd. In de zestiger jaren maakte Cavaliere melding van de preferentiële gevoeligheid van tumor cellen voor warmte, op grond waarvan hyperthermie aan de regionale perfusie techniek werd toegevoegd. Centraal bij de ILP staat dat concentraties tot 15 á 20 keer de maximale systemisch getolereerde concentratie van chemotherapeutica bereikt kunnen worden, aangezien vitale organen buiten de geperfundeerde circulatie liggen. Ten tijde van de introductie, werd de geïsoleerde ledemaat perfusie gebruikt voor lokaal uitgebreide melanomen, tumor recidieven en/of intransit metastasen. Na verloop van tijd werden ledemaat perfusies ook ingezet in de adjuvante behandeling van melanomen van extremiteiten. Een gerandomiseerde multi-center onderzoek onder EORTC auspiciën toonde geen voordeel aan van hyperthermische geïsoleerde ledemaat perfusies in de adjuvante behandeling voor patiënten met een hoog-risico stadium I melanoom (>1,5 mm Breslow dikte). Daarentegen is de geïsoleerde ledemaat perfusie met melfalan een erkende behandeling voor intransit metastasen en/of recidieven bij patiënten die niet in aanmerking komen voor een lokale behandelingvorm als chirurgie, cryotherapie of laserbehandeling. Ook andere chemotherapeutische middelen zijn gebruikt bij de perfusie van melanomen, maar deze hebben inferieure anti-tumor activiteit tegen veelal hogere toxiciteit. Gedurende de afgelopen veertig jaar heeft een scala aan alternatieve middelen de revue gepasseerd, waaronder dacarbazide (DTIC), actinomycine-D, thiotepa, mitomycine-C, doxorubicine en meer recent, cisplatine. De meerderheid van deze cytostatica was óf ineffectief, óf de responsduur was kort, óf lokale toxiciteit verhinderde verdere toepassing. Zo heeft doxorubicine wél

antitumor activiteit maar ook een scleroserend effect op de arteriën van het ledemaat, terwijl cisplatine te neurotoxisch is. Eind negentiger jaren werd TNFα in de perfusie geïntroduceerd door Lejeune, in combinatie met melfalan, in de behandeling van lokaal uitgebreide melanomen en sarcomen van ledematen. Uit een gerandomiseerd onderzoek tussen melfalan alleen en TNFα met melfalan voor patiënten met geavanceerde melanoom, bleek de toevoeging van TNFα geen duidelijk voordeel te brengen. Bij patiënten met een beperkte tumoruitbreiding of met kleine tumoren maakt het voor de respons geen verschil of TNFα met melfalan of melfalan alleen wordt gebruikt, terwijl de combinatie TNFα en melfalan wél een betere tumor respons geeft bij patiënten met lokaal uitgebreide melanomen. Vanwege deze bevindingen wordt melfalan gebruikt bij melanoom-perfusies en wordt alleen gecombineerd met TNFα in geval van uitgebreide ziekte óf bij recidieven na voorafgaande melfalan-perfusies.

In **hoofdstuk 3** wordt de effectiviteit en toxiciteit van carboplatine beschreven, een afgeleide van cisplatine, toegepast bij de perfusie van lokale recidieven en/of intransit metastasen van melanomen of lokaal uitgebreide weke-delen sarcomen. Drie patiënten, twee met lokaal uitgebreide melanomen en één patiënt met een laaggraadig liposarcoom van het been, werden behandeld met ILP onder milde hyperthermie (39-40°C) met 125 mg carboplatin per liter geperfundeerd ledemaatvolume. Er werd geen systemische toxiciteit waargenomen. Lokale toxiciteit bestond uit postperfusie oedeem en werd bij alle drie patiënten geobserveerd, maar verdween binnen twee weken. Persisterende lokale neuropathie werd geobserveerd bij alle drie patiënten en bij twee van hen werd dit geobjectiveerd met electromyografie en zenuwgeleidingsonderzoek. Ernstige senso-motorische neuropathie was aanwezig, vooral in de peroneale en surale zenuwen van de geperfundeerde ledematen. Bij pharmacokinetische analyse bleek de carboplatin-concentratie in de huid veel hoger dan in spierweefsel. De twee patiënten met een melanoom hadden een volledige tumor respons, maar beiden kregen een lokaal recidief binnen 12 maanden na popliteale en femorale perfusie. De derde patiënt onderging excisie van het sarcoom, acht weken na de perfusie. Maar bij histopathologisch onderzoek van het resectiepreparaat werd nog 50% vitaal tumorweefsel waargenomen. Eén patiënt met een melanoom en de patiënt met het sarcoom overleden ten gevolge van longmetastasen na respectievelijk 56

en 31 maanden na de perfusie behandeling. De tweede patiënt met een melanoom was in leven na 95 maanden na perfusie. Lokale neurotoxiciteit staat verdere toepassing van carboplatine bij ILP in de weg.

Met de komst van recombinant TNFα begin jaren negentig werd de effectiviteit van ILP voor patiënten met melanomen en vooral weke-delen sarcomen verbeterd. Echter, met lekkage van het TNFα naar de systemische circulatie traden ernstiger bijwerkingen op dan voorheen met het gebruik van melfalan alleen. Een lekkage van 1% TNFα kan reeds tot hypotensie leiden en een lekkage van 10% kan een potentieel lethaal beeld van septische shock induceren. Helaas is volledige isolatie van het ledemaat niet altijd mogelijk vanwege anatomische variaties en/of technische redenen. Daarom kan een geringe mate van lekkage niet altijd worden voorkomen en is continue lekkagemeting tijdens de perfusie procedure van het grootse belang. In de periode 1977-1991 werden 331 melfalan perfusies uitgevoerd in het GUMC, met een mediane lekkage van 8,0% (spreiding 0-30%).

In **hoofdstuk 4** wordt een overzicht gegeven van de gemeten lekkagepercentages naar de systemische circulatie met behulp van radioactieve tracers in de patiënten die geperfundeerd werden vanaf 1991. Het jaar 1991 valt ook samen met het moment van introductie van het uiterst toxische TNFα aan de ILP procedure. Het doel van dit onderzoek was om verschillen in de mate van lekkage tussen de periode 1977-1991 en 1991-2000 te achterhalen en daarvoor verklaringen te vinden. Honderd-negentien patiënten ondergingen ILP, hoofdzakelijk voor lokale recidieven van melanomen en voor lokaal uitgebreide weke-delen sarcomen. ILP werd verricht met melfalan alleen (33%) of in combinatie met TNFα (65%). Er werden 67 iliacale, 12 femorale, 25 popliteale en 15 axillaire perfusies uitgevoerd. Lekkage naar de systemische circulatie werd continu gemeten met behulp van ^{131}I-albumine via een stralingsmeter die vast boven het hart was geplaatst. Met een mediane lekkage van 2,7% (spreiding 0-21%) was er sprake van een significante daling ten opzichte van de voorgaande periode 1977-1991, met destijds 8,0% (0-30%) lekkage (P<0,05). Een significant statistisch verschil was er ook voor het niveau van de perfusies, waarbij iliacale en femorale perfusies een sterkere mate van lekkage vertoonden dan axillaire en popliteale perfusies (P<0,05). Bijkomstig bleek dat wanneer TNFα was gebruikt, er significant minder lekkage was ten opzichte van de perfusies met melfalan alleen (P<0,05).

150

We stelden vast dat tegenwoordig minder lekkage wordt bereikt in vergelijking met de periode dat alleen melfalan werd gebruikt. Bekend met de gevaren van TNFα lekkage, continue uitwendige lekkage-meting met ^{131}I-albumine als belangrijkste radioactieve tracer, bloedstroom-regulatie in de perfusie circulatie en regulatie van de bloeddruk in de patiënt zijn allen belangrijke oorzaken voor deze verbetering.

❑   Ongebruikelijke aspecten van melanomen

Zwangerschap

Melanomen worden frequent gediagnostiseerd bij jong volwassenen en een aanzienlijk deel van hen zijn vrouwen in de fertiele levensfase. De exacte incidentie van melanomen gedurende zwangerschap is niet bekend. Er wordt gesuggereerd dat zwangere vrouwen een slechter prognose hebben ten gevolge van een agressiever biologisch gedrag van het melanoom door hormonale of reproductieve factoren. **Hoofdstuk 5** heeft ten doel om het lange-termijn effecten van zwangerschap op de progressie van stadium I/II melanomen te evalueren. Van 1965 tot 2001 werden 46 zwangere vrouwen behandeld voor een stadium I/II melanoom. Deze patiënten werden vergeleken met een groep van 368 niet-zwangere patiënten met stadium I/II tumoren, gecorrigeerd voor leeftijd en geslacht. Patiënten werden gestadieerd volgens het 2002 AJCC TNM systeem. De 10-jaars ziektevrije overleving (DFS) en de 10-jaars overleving (OS) werden berekend met de logistische regressie methode. De mediane leeftijd voor de zwangeren was 30 (spreiding 18-46) jaar en 36 (spreiding 17-45) jaar voor de niet-zwangeren. De mediane follow-up bedroeg 109 maanden. Zwangeren presenteerden zich vaker met dikkere melanomen (mediaan 2,0 mm versus 1,7 mm, *ns*). Er werd geen verschil in tumor-localisatie, histologisch subtype, tumor ulceratie en vasculaire invasie waargenomen tussen de zwangere en niet-zwangere groep. Evenmin was er een statistisch significant verschil in 10-jaars DFS en 10-jaars OS tussen beide groepen. De 10-jaars DFS voor zwangeren en niet-zwangeren was respectievelijk 88% en 86% bij stadium I ziekte, en 67% versus 73% voor stadium II patiënten. De 10 jaars OS was respectievelijk 94% versus 90% voor stadium I en 82% versus 81% voor stadium II. Concluderend wordt gesteld dat

zwangerschap de lange-termijn overleving van patiënten met melanomen in een beperkt ziektestadium niet ongunstig beïnvloedt.

Kinder en juveniele melanomen

Het ontstaan van melanomen vóór de puberteit is zeldzaam, maar de incidentie stijgt met de leeftijd. De sterkste stijging in incidentie wordt waargenomen bij patiënten boven de 65 jaar, tegen een meer gematigde stijging bij jongeren. Circa 1 tot 4% van alle nieuwe gevallen van melanomen wordt gevonden bij patiënten onder de leeftijd van 20 jaar en slechts 0,3% bij patiënten jonger dan 14 jaar. In **hoofdstuk 6** worden het kinderleeftijd en juveniele melanoom geanalyseerd, die in het GUMC zijn behandeld; de klinische karakteristieken, prognostische factoren, ziektevrije en totale overleving werden vergeleken met tumoren bij volwassenen. Gedurende 1965 tot 2003 werden 55 patiënten onder de 19 jaar behandeld voor een cutaan melanoom. Vergelijking vond plaats met een controlegroep van 972 volwassen patiënten. Patiënten werden gestadieerd volgens het 2002 AJCC TNM melanoom stadiëringssysteem. De 10-jaars DFS en de 10-jaars OS werden berekend met logistische regressie; onafhankelijke prognostische factoren voor OS en DFS werden aanvullend geanalyseerd. Zes patiënten waren jonger dan 12 jaar en daarmee uitgesloten van statistische analyse. De mediane leeftijd voor juveniele patiënten was 17 jaar, tegen 49 jaar voor de controlegroep. Er was een mediane follow-up van 92 maanden. Beide groepen hadden een vergelijkbare Breslow dikte (juveniele groep 1,6 mm versus volwassen groep 2,0 mm; P=0.075). Juveniele patiënten presenteerden zich vaker met lokaal uitgebreide melanomen (P<0,01). Leeftijd, mitose ratio, ulceratie, Breslow dikte, mannelijk geslacht en stadium bij diagnose bleken allen onafhankelijke prognostische factoren in de volwassen groep. Bij de juveniele groep daarentegen was alleen stadium bij diagnose een significante factor. Er werd geen statistisch verschil in de 10-jaars DFS en 10-jaars OS gevonden tussen de twee groepen. De 10-jaars DFS voor de juveniele en de volwassen groep was respectievelijk 64% en 71% voor stadium I/II, en 45% en 40% voor stadium III. De 10-jaars OS was respectievelijk 79% en 83% voor stadium I/II en 50% en 50% voor stadium III. Er is geen verschil in 10-jaars DFS en OS tussen de twee groepen, al presenteerden de juveniele patiënten zich wel vaker in een verder gevorderd ziektestadium.

Behalve stadium bij diagnose, lijken de tegenwoordig toepaste prognostische factoren niet te gelden voor juveniele patiënten. Welke prognostische factoren bij hen spelen is onbekend, zodat aanvullend onderzoek nodig is om factoren die het beloop bepalen bij juveniele patiënten te identificeren.

Hersenmetastasen van cutane hoofd hals melanomen

Er is een stijgende incidentie van het aantal cutane melanomen (CM), waarvan 10-20% in het hoofd/hals gebied optreedt. In **hoofdstuk 7** worden de incidentie van hersenmetastasen, risicofactoren en uitkomst geanalyseerd van melanomen die zich in het hoofd/hals gebied presenteren. In de periode 1965 tot 2000 werden 324 patiënten (152 vrouwen en 172 mannen) behandeld in het GUMC voor een CM van het hoofd/hals gebied. Patiënten werden gestadieerd volgens het 2002 AJCC melanomen stadiëringgssysteem. Om risicofactoren te identificeren, werd een *matched control* analyse verricht. Dit werd gedaan met gebruikmaking kruistabellen, chi-kwadraat toetsen en logistische regressie analyse. Zesentwintig patiënten (8%) met hoofd/hals tumoren in vergelijking met 5,2% (betrouwbaarheidsinterval 0,058- 0,108, $P<0,05$) extremiteit/romp tumoren ontwikkelden hersenmetastasen. De 26 hoofd/hals patiënten (4 stadium I, 10 stadium II, en 12 stadium III) hadden een mediane leeftijd van 46 jaar en ontwikkelden hersenmetastasen na een mediane periode van 24 maanden. De mediane Breslow dikte van hun tumoren bedroeg 3,3 mm. Behandeling bestond uit toediening van steroïden, chirurgie, bestraling, chemotherapie of een combinatie hiervan. De mediane overleving gerekend vanaf het ontstaan van hersenmetastasen was 2,4 maanden, met een éénjaars overleving van 15%. Als risicofactoren voor het ontstaan van hersenmetastasen vanuit hoofd/hals melanomen kwamen jongere leeftijd, mannelijk geslacht, Breslow dikte meer dan 4 mm en mitose ratio naar voren. Hoofd/hals CM geven in 8% aanleiding tot hersenmetastasering tegen 5,2% van extremiteit/romp melanomen; het ontstaan van hersenmetastasen is geassocieerd met bovenstaande prognostische factoren. De prognose van de patiënten met hersenmetastasendeze is met de huidige beschikbare behandelingsmogelijkheden nog steeds slecht. Metastasectomie en adjuvante radiotherapie voor solitaire hersenmetastasen kunnen in geselecteerde gevallen de overleving verlengen.

153

❑ Conclusies

- Geïsoleerde hyperthermische ledemaat perfusie met melfalan heeft zich ontwikkeld tot een volwaardige palliatieve behandelingsmodaliteit voor intransit metastasen en/of recidieven bij patiënten die niet voor een lokale chirurgische ingreep in aanmerking komen.
- Geïsoleerde hyperthermische ledemaat perfusie met carboplatine in de behandeling van lokaal uitgebreide melanomen gaat gepaard met ernstige neurotoxiciteit van het behandelde ledemaat.
- Continue uitwendige monitoring met ^{131}I-albumine als belangrijkste isotoop is een betrouwbare methode om lekkage van het perfusaat naar de systemische circulatie te detecteren.
- Zwangerschap heeft geen ongunstige invloed op de lange-termijn uitkomst van patiënten met een primair stadium I of II melanoom.
- De lange-termijn uitkomst voor kinderen/juvenielen met een melanoom is vergelijkbaar met die van volwassen patiënten. Echter, behalve het ziektestadium ten tijde van de presentatie van de ziekte, zijn de huidig bekende prognostische factoren niet van toepassing op kinderen/juvenielen met een melanoom.
- Er is een verhoogde incidentie van hersenmetastasen afkomstig van cutane hoofd/hals melanomen ten opzichte van cutane melanomen van romp en extremiteiten.

154

❑ **Vooruitblik**

Hoewel conventionele ILP een effectieve modaliteit is in de behandeling van recidief melanomen van arm of been, blijft het een complexe en invasieve procedure. Er geldt een strikte patiënten-selectie zodat veel patiënten niet behandeld kunnen worden. Zo worden vaak patiënten met afstandsmetasasen naast een recidief aan het ledemaat, en patiënten met ernstige co-morbiditeit veelal uitgesloten van ILP behandeling. In 1994 werd door Thompson van de Sydney melanoma unit een gesimplificeerde methodiek van ILP, namelijk geïsoleerde ledemaat infusie (ILI), geïntroduceerd. Deze nieuwe techniek is feitelijk een ILP met lage stroomsnelheid die onder hypoxische condities wordt uitgevoerd met klein-caliber arteriële en veneuze katheters, die percutaan worden opgevoerd via de contralaterale lies. Vanwege de lage drukken die hiermee worden gegenereerd, is een pneumatische tourniquet afdoende om systemische lekkage van de gebruikte chemotherapeutische middelen te voorkomen. Het is bij deze techniek echter niet mogelijk om de temperatuur in arm of been boven de 38,5 C op te voeren. De ILI procedure heeft mogelijk een equivalente effectiviteit ten opzichte van ILP en zou toegepast kunne worden bij de oudere en medisch gecompromitteerde patiënt. Bijkomend voordeel is dat de ILI-procedure herhaaldelijk kan worden uitgevoerd en vormen afstandsmetastasen geen contra-indicatie.

De ziekte melanoom is anno 2004 nog steeds onvoorstelbaar en onvoorspelbaar De enige kans op genezing biedt radicale chirurgische verwijdering(en) van melano(o)m(en) en van de eventuele metastasen. De komende jaren zal door de afdeling Chirurgische Oncologie van het GUMC en andere instituten de waarde van de schildwachtklierbiopsie bij het melanoom stadium I (MSLT I en II studie), de whole-body CT scan en whole-body PET scan (OMSPECT studie) bij de stadiering van het locoregionaal gemetastaseerde melanoom (stadium III) en de behandeling met een polyvalent melanoom vaccin bij het gemetastaseerde melanoom (stadium III en IV) onderzocht worden. Daarnaast zal onderzoek naar de waarde van follow-up bij het melanoom (MELFON studie) geinitieerd worden.

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158

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Curriculum Vitae

The author of this thesis was born on March 17, 1969, in Willemstad Curacao. In 1987 he graduated from high-school (VWO, Colegio Arubano, Aruba) and went to study Biochemistry and Mathematics (Boston College, Massachusetts, USA) for which he received a Bachelors degree in 1992. That same year he moved to Groningen, The Netherlands to study Medicine at the University of Groningen which was completed in 1998. In 1998 he worked as an assistant resident for one year at the Department of Surgery of the Groningen University Hospital. The basis of the thesis was made during a research year in 1999 at the Division of Surgical oncology. Between September 2000 and September 2003 he worked as a resident in general surgery at the Groningen University Hospital. (head: Prof. dr. R. van Schilfgaarde/Prof. dr. H.J. ten Duis). Currently, he is completing his residency in general surgery at the Medisch Spectrum Twente (head: Prof. dr. P.A.M. Vierhout/Dr. W.J.B. Mastboom) in Enschede, The Netherlands.

1. Geïsoleerde hyperthermische ledemaatperfusie met melfalan heeft zich ontwikkeld tot een volwaardige palliatieve behandeling van intransit metastasen en/of recidief melanomen die niet voor een lokale chirurgische ingreep in aanmerking komen.

2. Geïsoleerde hyperthermische ledemaatperfusie met carboplatine gaat gepaard met ernstige neurotoxiciteit van de behandelde extremiteit.

3. Het optreden van systemische toxiciteit bij perfusie van een extremiteit met TNFα heeft ertoe geleid dat chirurgen bij het uitvoeren van de perfusie meer bedacht zijn op het voorkómen van lekkage.

4. Continue uitwendige controle met ^{131}I-albumine als belangrijkste isotoop is een betrouwbare methode om lekkage van het perfusaat naar de systemische circulatie op te sporen.

5. Het bestaan van een zwangerschap bij de presentatie van een primair stadium I of II melanoom van de huid heeft geen ongunstige invloed op de overleving van de moeder.

6. In vergelijking met volwassenen is er bij patiënten jonger dan 19 jaar met een melanoom van de huid vaker sprake van een stadium III bij presentatie.

7. De keuze van de Nederlandse Antillen en Aruba voor UPG (Ultraperifeer Gebied) of LGO (Landen en Gebieden Overzee) moet niet de uitkomst zijn van een boekhoudkundige exercitie. Het is een politieke keuze, waarbij het van belang is zich af te vragen wat voor land Aruba en de Antillen op de lange termijn willen zijn in relatie tot de rest van de wereld.

8. Veel van de belangrijke culturele overeenkomsten en verschillen tussen mensen in de hedendaagse wereld zijn eerder verbonden met het beroep dan met de nationaliteit.

9. Democracy must, in essence, mean the art and science of mobilizing the entire physical, economic and spiritual resources of all the various sections of the people in the service of the common good of all.
   (Mahatma Gandhi, Mohandas Karamchad Gandhi, 1869-1948)

10. Evenals de primitieve tovenaar wordt de deskundige aangemoedigd zich bezig te houden met zijn geheimzinnige berekeningen, en blijft geloofwaardig ook als de gebeurtenissen hem in het ongelijk stellen.
    (Gareth Morgan, Images of Organization, Sage Publications 1986)

11. Until we are able to control human overpopulation, any species that competes with 'Homo sapiens' for space and food is doomed.
    (Karla Kellenberger, Time Magazine,Volume 164, nummer 11, September 13, 2004)

Groningen, 26 januari 2005