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Angiographic Response of Locally Advanced Soft-Tissue Sarcoma Following Hyperthermic Isolated Limb Perfusion with Tumor Necrosis Factor

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Background: Hyperthermic isolated limb perfusion (HILP) with tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and melphalan is associated with a dramatic anti-tumor effect in which the neo-vascularization of the tumor is supposed to be the major target. The aim of the present study was to correlate the angiographic findings with the pathological response in patients undergoing HILP for locally advanced soft-tissue sarcoma.

Patients and Methods: Twenty-five patients, 14 male and 11 female, mean age 47 years (range 18–80) were studied. Angiographies were performed before and a median period of 7 weeks (range 4–14 weeks) after HILP. Eight weeks after perfusion, the residual tumor mass was resected and pathologically examined. The changes in tumor vascularization after treatment were scored and compared with the pathological response.

Results: All baseline angiograms showed a hypervascular tumor. After HILP, a normal angiography result (NA) was observed in 18 patients (72%) and an abnormal angiography result (AA) was observed in seven patients (28%). All patients with an NA showed a pathologically complete response (pCR) or a pathological partial response with >90% necrosis of the tumor. Of seven patients with an AA, pathological examination showed a pCR in one patient, 10–50% viable tumor volume in four patients, and no pathological response after perfusion in two patients. A good correlation was seen between angiographic and pathological classification ($p < 0.001$).

Conclusion: An angiography performed after hyperthermic isolated limb perfusion with TNF-α and melphalan provides a good indication, regardless of whether a good pathological response is expected.

Key Words: Hyperthermic isolated limb perfusion—Angiography—Sarcoma—Tumor necrosis factor.

Soft-tissue sarcomas are rare. They account for <1% of all malignant tumors in adults (1). Sixty percent of soft-tissue sarcomas are localized in the extremities and are often large at time of diagnosis (2). Radical surgical resection of locally advanced or recurrent soft-tissue sarcomas of the extremities is generally only feasible with an amputation or ex-articulation of the affected limb.

During the past two decades, progress was made in the diagnostic imaging and histological classification of soft-tissue sarcomas. Computer tomography and magnetic resonance imaging improved insight in the local growth pattern of the tumor, whereas immunohistochemistry techniques and electron microscopy improved the histological diagnosis (3,4). Combined treatment modalities, consisting of surgery with pre- or postoperative radiotherapy and/or chemotherapy improved the local control rate, resulting in a limb salvage rate of ~90% with a local

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recurrence rate of <15%. Still, 40% of the patients with high-grade malignant lesions die from metastatic disease (5). Preservation of the extremity and good limb function have become all the more important because amputations do not improve survival rates (6).

The technique of hyperthermic isolated limb perfusion (HILP) was first described by Creech et al. in 1957 and is most commonly used to treat patients with recurrent melanoma, satellitosis, or in-transit metastases of the extremity with melphalan as the standard perfusion agent (7–11). HILP for soft-tissue sarcomas is an attractive mode of therapy considering that 15–20 times higher regional cytostatic concentrations can be reached compared with systemic administration with minimal systemic toxicity (12). However, the results of HILP for extremity soft-tissue sarcomas with various cytostatic agents are not impressive (13–15). In search of more effective treatment modalities for extremity soft-tissue sarcomas, Lejeune et al. investigated the addition of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in the limb perfusion setting, which resulted in a high rate of complete remission in patients with extremity soft-tissue sarcomas (16). Since 1991, patients with locally advanced extremity soft-tissue sarcomas have been treated at the Groningen University Hospital by HILP, with TNF-α, IFN-γ, and melphalan as perfusion agents, followed by delayed excision. Because the tumor vascularization, particularly the endothelial cells, are supposed to play a key role in the antitumor effect of TNF-α, the aim of the present study was to investigate the angiographic changes in patients undergoing TNF-α perfusion for locally advanced soft-tissue sarcoma and to relate the angiographic changes with the histological response of the resected specimen in a prospective manner.

PATIENTS AND METHODS

During July 1991 to January 1995, 25 consecutive patients with biopsy-proven locally advanced soft-tissue sarcoma were treated with HILP followed by delayed limb salvage surgery. The pathological diagnosis and malignancy grade of the tumor according to Trojani (17) are summarized in Table 1. Fourteen patients were male, 11 female. The mean age was 47 years (range 18–80). Informed consent was obtained from each patient. The lesions were localized on the lower extremity in 23 patients (92%) and on the upper extremity in the remaining two patients (8%). Twenty-two patients presented with a newly diagnosed solitary soft-tissue sarcoma (mean tumor size 13 × 9 cm), and three patients presented with multiple local recurrences (i.e., two or three lesions with a minimal size of 3 × 4 cm and a maximum size of 4 × 8 cm.

The perfusion technique used at the Groningen University Hospital is based on the previously described technique developed by Creech and Krementz (7,18). Briefly, after ligation of all collateral vessels and heparinization of the patient with 3.3 mg heparin/kg body weight (Thromboliquine, Organon BV, Oss, The Netherlands), the major limb vessels are cannulated and connected to an extracorporeal circuit. Collateral vessels are ligated and a tourniquet is applied to compress the remaining minor vessels. Perfusion is performed for 90 min under mild hyperthermia (39–40°C) and physiologically optimal conditions (19). At the start of perfusion, 3 mg (upper extremity) or 4 mg (lower extremity) recombinant TNF-α (Boehringer, Ingelheim, Germany) is injected as a bolus into the arterial line. Melphalan (Burroughs Wellcome, London, England) is administered 30 min later as a 10 mg/L extremity volume (leg) or 13 mg/L extremity volume (arm) (20). Leakage of the perfusion circuit to the systemic circulation is measured with radiolabeled iodide and technetium (21). Because all perfusions were performed in a phase II clinical trial, the initial 11 patients in this study also received a dose of 0.2 mg recombinant IFN-γ (Boehringer Ingelheim, Germany) subcutaneously 1 and 2 days before perfusion, followed by 0.2 mg IFN-γ injected into the arterial line at the start of perfusion. The final 14 patients in this study did not receive the IFN-γ. This alteration in treatment schedule was due to the decision of the trial commission to investigate the additional effect of IFN-γ in the perfusion regimen, whereas the angiographic study was still in progress. Perfusion was performed via the iliac artery in 13 patients (52%), the popliteal artery in 10 patients (40%), and the axilla artery in the remaining two patients (8%) for tumor localization in the upper extremity.

Selective arterial angiography of the affected extremity was performed using the Seldinger technique through the common femoral artery under local anesthesia with low-osmolar contrast media. The first angiography was performed before tumor biopsy and perfusion, the second after a median period of 7 weeks (range 4–14 weeks) after perfu-

TABLE 1. *Patient characteristics*

Patient	Sex	Age (yr)	Arm/leg	Histology	Malignancy grade	Perfusion agents	Response	
							Angiographic	Pathological
1	F	39	L	Myxoid liposarcoma	I	TNF, IFN, melphalan	AA	pCR
2	F	44	L	Myxoid liposarcoma	I	TNF, IFN, melphalan	AA	pPR50%
3	M	44	L	Myxoid liposarcoma	I	TNF, melphalan	NA	pPR90%
4	F	60	L	Leiomyosarcoma	II	TNF, IFN, melphalan	NA	pPR90%
5	F	61	A	MFH	II	TNF, IFN, melphalan	NA	pPR90%
6	M	62	L	Malignant schwannoma	II	TNF, IFN, melphalan	AA	pNC
7	M	18	L	Chondrosarcoma	II	TNF, IFN, melphalan	NA	pPR90%
8	M	53	L	MFH	II	TNF, IFN, melphalan	NA	pPR90%
9	F	26	L	Synovial sarcoma	II	TNF, melphalan	NA	pPR90%
10	M	64	L	MFH	II	TNF, melphalan	AA	pPR50%
11	M	25	L	Synovial sarcoma	II	TNF, melphalan	NA	pPR90%
12	F	53	L	NOS sarcoma	II	TNF, melphalan	NA	pCR
13	F	18	L	Rhabdomyosarcoma	III	TNF, IFN, melphalan	NA	pCR
14	F	50	L	Myxoid liposarcoma	III	TNF, IFN, melphalan	NA	pCR
15	M	28	L	Malignant schwannoma	III	TNF, IFN, melphalan	NA	pPR90%
16	M	43	L	Synovial sarcoma	III	TNF, melphalan	NA	pPR90%
17	F	56	L	PNET	III	TNF, melphalan	NA	pCR
18	M	80	L	NOS sarcoma	III	TNF, melphalan	AA	pPR50%
19	M	56	L	MFH	III	TNF, melphalan	NA	pCR
20	F	40	L	MFH	III	TNF, melphalan	NA	pCR
21	F	39	A	Synovial sarcoma	III	TNF, melphalan	NA	pCR
22	M	74	L	NOS sarcoma	III	TNF, melphalan	NA	pCR
23	M	50	L	MHP	III	TNF, melphalan	AA	pNC
24	M	51	L	MHP	III	TNF, melphalan	AA	pPR50%
25	M	42	L	Clear cell sarcoma	III	TNF, IFN, melphalan	NA	pCR

MFH, malignant fibrous histiocytoma; NOS, not otherwise specified sarcoma; PNET, peripheral neuroepithelioma; MHP, malignant hemangiopericytoma; TNF, tumor necrosis factor; IFN, interferon; NA, normal angiography; AA, abnormal angiography after perfusion.

sion. No local or systemic complications were encountered. The angiographs were evaluated retrospectively by a radiologist (E.L.M.) without knowledge of the histological findings or clinical course of the disease. The findings on the pre- and postperfusion angiograms were compared, and changes in tumor vascularization were classified. An angiographic response was defined as a complete disappearance of tumor vascularity and tumor stain (NA, normal angiography result). No response was defined as persistence of tumor vascularity and tumor stain (AA, abnormal angiography result).

Approximately 8 weeks after perfusion, the residual tumor mass was excised. The resected tumor specimen was measured in three dimensions and the percentage of necrosis estimated. Representative tumor sections were taken, encompassing macroscopically different tumor areas, including necrosis. As a rule, one section per centimeter largest diameter (minimum of three) was taken. Based on an integration of gross and microscopic findings, a final estimate of the percentages of viable and necrotic or regressive tumor was made. The tumor biopsy and resected surgical specimen were evaluated by the same pathologist (W.M.M.). A four-grade scale (Table 2) was applied in assessing the pathological response to TNF-α perfusion. Scores I and II were considered responders, and scores III and IV were classified as nonresponders. Finally the angiographic score was compared with the pathological score of the resected tumor specimen.

Besides the angiographic and pathological responses, tumor size reduction of the tumor also was scored. Before perfusion, tumor size was determined via magnetic resonance imaging in three dimensions and after perfusion by measuring the re-

TABLE 2. *Histological score of tumor response to TNF perfusion*

I. Pathololgically complete response	No viable tumor cells
II. Pathololgically partial response 90%	Isolated viable tumor cells or <10% viable tumor
III. Pathololgically partial response 50%	Viable tumor accounting for 10–50% of tumor volume
IV. Pathologically no change	Viable tumor accounting for >50% of tumor volume

sected tumor specimen three-dimensionally. Size reduction was expressed in the percentage of reduction with regard to the size before perfusion.

Statistical procedures included Fisher's exact test. A probability value $p < 0.05$ was considered significant. Graph Pad Prism version 2.0 for Windows statistical software was used.

RESULTS

The baseline angiograms obtained before incisional biopsy and TNF-α perfusion showed that all 25 patients had an abundance of tumor neovascularity and stain, a typical hypervascular pattern of tumor. Table 1 lists the results of the individual patients. Reduction in tumor size after perfusion and angiographic devascularization were observed in 18 patients (72% NA). Figure 1 gives an illustration of a complete normalization of the neovascular pattern of the tumor after perfusion (patient 13). Histologically, nine of these 18 NA patients had a devitalized tumor (pCR), and nine patients had remaining isolated viable tumor cells or <10% viable tumor volume (pPR90%). Of the remaining patients, seven showed persisting tumor vascularity (28% AA). Pathological examination of the residual tumor mass in these patients showed a complete response in one patient, 10–50% viable tumor volume in four patients, and no pathological response to TNF-α perfusion in two patients. The results of the post-perfusion angiograms compared with the histological findings are summarized in Table 3. When patients were divided into the clinically relevant subgroup of responders and nonresponders, correlation demonstrated 18 true-positive cases (with no residual tumor vascularity and therefore responders), zero false-positive cases, six true-negative cases (with residual tumor vascularity and therefore nonresponders), and one false-negative case ($p < 0.001$). This yielded a sensitivity (percentage of nonresponders identified) of 95% and a specificity (percentage of responders identified) of 100%.

TABLE 3. *Angiographic–histologic relationship of tumor response after TNF perfusion*

	Histologic score				
	Responders		Nonresponders		
Angiographic score	pCR	pPR90%	pPR50%	pNC	Total
NA	9	9			18
AA	1		4	2	7
Total	10	9	4	2	25

The relationship between the malignancy grade of the tumor and the angiographic changes in tumor vascularization and the pathological response after perfusion were analyzed as well. For the 13 patients with malignancy grade III sarcomas, the results on angiography were 10 patients with an NA (77%) and three with an AA (23%). Of nine patients with grade II sarcomas, seven scored an NA (78%) and two an AA (22%). For the three patients with grade I sarcomas, angiography showed one NA (33%) and two AAs (67%). No statistical relationship was observed between malignancy grade and angiographic score after TNF-α perfusion.

A,B

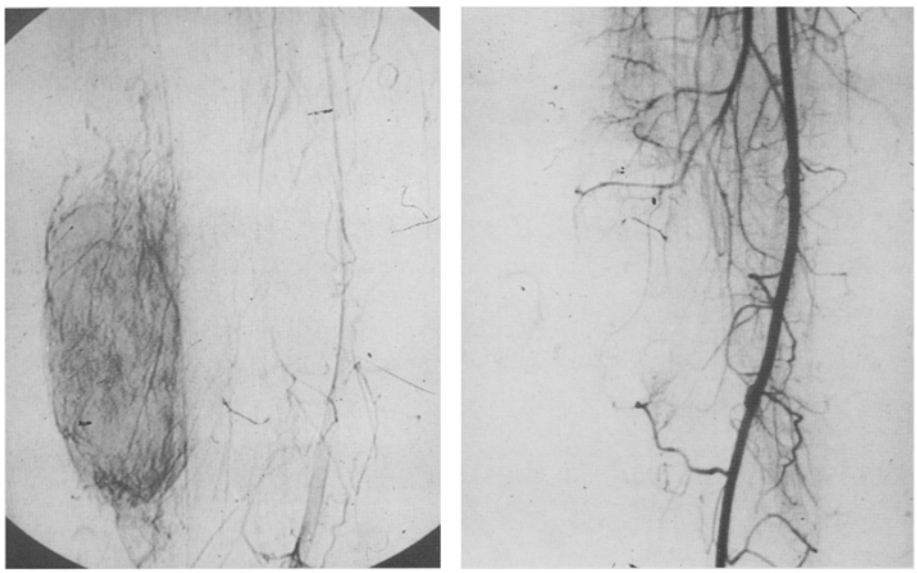

FIG. 1. A: Angiography of a rhabdomyosarcoma of the right thigh in an 18-year-old woman before TNF-α perfusion. The tumor shows an abnormal vascular pattern with tumor blush, cork screws, and sclerosis. B: Angiography in the same patient 7 weeks after TNF-α perfusion, demonstrating a complete normalization of the previously abnormal vascular pattern.

For the 13 patients with malignancy grade III sarcomas, the pathological response was eight with pCR, two with pPR90%, two with pPR50%, and one with pNC. Of nine patients with grade II sarcomas the response was one with pCR, six with pPR90%, one with pPR50%, and one with pNC. For the three patients with grade I sarcomas, the histological response was pCR, pPR90%, and pPR50% in one patient each. A pathological complete response after perfusion was observed more often in malignancy grade III sarcomas compared with malignancy grade I and II sarcomas (62% vs. 17%) ($p < 0.05$).

Mean three-dimensional size reduction of the tumor after perfusion was 35%, including five patients in whom the tumor did not change in size. These five included two patients who did not show a pathological response after perfusion, one with a pCR, one with a pPR90%, and one with a pPR50%. Forty-eight percent size reduction was observed in those patients in whom the tumor became smaller. None of the tumors showed any progression in size after perfusion.

DISCUSSION

HILP with TNF-α and melphalan is an effective therapeutic approach in patients with locoregional disease such as recurrent melanoma, in-transit metastasis of melanoma, and recurrent or locally advanced soft-tissue sarcomas (22,23). Recently, Renard et al. demonstrated in a morphological and immunohistochemical study of human tumors similar in design to the present study that tumor microvascularization is the primary target for TNF-α, resulting in coagulative and hemorrhagic necrosis of the tumors (24). These findings were in concordance with data of murine experiments (25). In the study of Renard et al., a key role is reserved for tumor-associated endothelial cells. TNF-α exposure leads to endothelial swelling with dendritic extension formation. As a result of the endothelial cell activation, induction, amplification, and expression of adhesion molecules takes place. These changes are observed within a few hours after perfusion with TNF-α and did not occur after perfusion with melphalan alone. Expression and upregulation of adhesion molecules subsequently leads to an increased homing of polymorphonuclear cells within 3 h in the tumors but not in normal skin. This TNF-α–induced cascade finally results in coagulative and hemorrhagic necrosis of the tumor observed within 3 h to 3 days after perfusion with TNF-α.

The present angiographic study demonstrates the specific destruction of tumor vessels after perfusion with TNF-α, leaving the normal vasculature unchanged. A complete normalization of the hypervascular pattern of the tumor after TNF-α perfusion was associated with complete or <10% viable tumor tissue on pathological analysis of the resected specimen. Remaining tumor vascularity after TNF-α perfusion was associated with >10% of viable tumor on pathological examination, except for one case in which a pathologically complete response was observed. Assessment of the angiographic response after perfusion demonstrated that 18 of the 19 pathological responders (95%) were correctly identified with angiography, and none of the six pathological nonresponders were falsely considered responders due to angiographic results. Only one (5%) of the 19 pathological responders was falsely considered a nonresponder due to angiographic findings. Therefore, persistent tumor vascularity after TNF-α perfusion was unlikely in the pathologic responders, and it was highly suggestive of a poor response. No relationship was observed between the malignancy grade of the tumor and the angiographic score. However, malignancy grade III sarcomas showed a pathologically complete response after perfusion more often compared with malignancy grade I and II sarcomas.

After perfusion, the tumor decreased in size in 20 patients by a mean of 48%, and in five patients no change in tumor size was observed. Although reduction in tumor size is an important factor to render locally advanced tumors resectable after perfusion, the observed clinical softening of the tumor consistency, which is seen within 3 days after perfusion, helps in distinguishing the margins from the surrounding normal tissues, facilitating resection. The devascularized tumor can be "peeled" out of the normal tissues.

CONCLUSION

Soft-tissue sarcomas present abnormal vascular patterns on angiography. An angiography performed after hyperthermic isolated regional perfusion with TNF-α gives a good indication regardless of whether a good pathological response is expected.

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