Value of continuous leakage monitoring with radioactive Iodine-131 labeled human serum albumin during hyperthermic isolated limb perfusion with TNF and melphalan

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Abstract

The aim of this study was to analyze the value of continuous leakage monitoring with radioactive Iodine-131 labeled human serum albumin (RISA) in patients treated with hyperthermic isolated limb perfusion (HILP) with tumor necrosis factor alpha (TNF) and melphalan. Forty-eight patients with melanoma (n = 14) or soft tissue sarcoma (n = 34) of an extremity underwent 51 perfusions. Perfusion was performed at the iliac level in 22 cases, at the popliteal level in 16 cases, at the femoral level in 7 cases and in 6 cases at the axillary level. Leakage rates, perfusion circuit and systemic levels of TNF, interleukin-6, C-reactive protein (CRP) were determined, as were systemic hematological and metabolic profiles and tumor response. The mean isotopically measured leakage was 2.9 % (95% confidence interval 2.0 – 3.8%, range 0-15.5%). Systemic leakage was ≤2% in 28 perfusions (55%) and >2% in 23 perfusions (45%). The correlation between the maximal monitored leakage and maximal systemic TNF levels was 0.7114 (p < 0.0001). The area under the curve (AUC) for TNF in the perfusion circuit, indicating the exposure of the perfused limb to TNF, was 18.7% lower in the >2% leakage group (p=0.0457). No significant differences in tumor response were found between groups. AUC for systemic TNF, indicating the exposure of the patient to TNF, was 18.1 times higher in the >2% leakage group (p<0.0001) resulting in a significant decrease in leucocyte and platelet count, hyperbilirubinemia, hypocholesterolemia and proteinemia. No beneficial effect of the systemically leaked TNF and melphalan was seen on the occurrence of distant metastasis during follow-up. There was a significant difference between perfusions performed at the iliac and femoral levels compared with leakage values at the popliteal level, p < 0.0001 and 0.0159 respectively. A good correlation between RISA leakage measurement and TNF exposure during and after HILP with TNF and melphalan was demonstrated. RISA leakage measurement serves as a good guide for the effectiveness of isolation during perfusion. If leakage exceeds the 2% limit during perfusion, less exposure of the tumor bearing limb to TNF, increased exposure of the patient systemic circulation to TNF, and more systemic side effects can be expected.

Introduction

Creech and Krementz developed isolated limb perfusion with chemotherapy for the treatment of extremity melanoma in humans in 1958. Stehlin et al modified the technique in 1969 to include hyperthermia. Since then, hyperthermic isolated limb perfusion (HILP) with different chemotherapeutic agents has been used by several institutes worldwide for the treatment of advanced extremity melanoma and soft tissue sarcoma. Recently, an international study comparing local excision and adjuvant
HILP with melphalan with wide excision only revealed a trend for a longer disease-free interval after HILP with melphalan but no benefit from HILP in terms of time to distant metastasis or survival. With the conclusion that prophylactic HILP with melphalan could not be recommended as an adjunct to standard surgery in high-risk primary limb melanoma, the indication for HILP is currently restricted to advanced melanoma and primarily irresectable soft tissue sarcoma. For these indications the addition of tumor necrosis factor alpha (TNF) to melphalan seems promising.

With the introduction of TNF, monitoring of leakage of the isolated circuit into the systemic circulation has been mandatory since TNF levels in the perfusion circuit are approximately 10 times the maximum tolerated systemic levels. If significant leakage occurs during HILP, the resultant TNF induced systemic inflammatory response syndrome (SIRS) could be fatal. Different methods for measurement of leakage are used. In the early days, Stehlin et al determined the amount of radioactive Iodine-131 labeled human serum albumin (RISA) through the use of blood samples from the systemic circulation, and calculated the leakage factor (LF). Although determination of blood samples takes time and is discontinuous, it is frequently used by other groups. To overcome these disadvantages, Stehlin and associates were the first to describe a method of continuous external leakage monitoring with RISA. Because of safety regulations, nuclear medicine techniques are not always allowed in the operation zoom. Another method, the measurement of Evans blue concentration in plasma by means of a spectral photometer, overcomes this problem. Two other groups introduced the use of handheld gamma detectors for leakage measurements; however, a great dependency was observed on the distance and angle from the source with this system.

Since 1991, patients with advanced melanoma or soft tissue sarcoma of the limbs, have been treated at the Groningen University Hospital by HILP with TNF, melphalan with or without interferon gamma (IFN) as perfusion agents, followed by delayed surgical excision. The aim of this study was to analyze the value of continuous leakage monitoring with RISA in patients treated with TNF perfusion with respect to systemic levels of TNF, interleukin (IL)-6, C-reactive protein (CRP) as well as hematological and metabolic profiles and tumor response.

**Patients and methods**

Forty-eight patients with melanoma (n = 14) or soft tissue sarcoma (n = 34) of the extremity underwent 51 perfusions with a combination of TNF and melphalan, with or without IFN. Twenty-one males and 27 females, with a median age of 54 years (range 18-80 years) were treated. Perfusion was performed at the iliac level in 22 cases (43%), at the popliteal level in 16 cases (31%), at the femoral level in 7 cases
(14%), and in 6 cases (12%) at the axillary level. All patients were treated after informed consent was obtained according to institutional guidelines.

**Perfusion Technique**

The perfusion technique employed at the Groningen University Hospital is based on the technique developed by Creech et al.\(^1\) and has been described in detail previously.\(^{15}\) Briefly, after ligation of all collateral vessels and heparinization of the patient with 3.3 mg heparin/kg bodyweight (Thromboliquine, Organon BV, Oss, the Netherlands), the axillary, iliac (internal iliac artery is temporarily closed), femoral, or popliteal vessels were cannulated and connected to an extracorporeal circuit. The perfused limb was wrapped in a thermal blanket to reduce heat loss. To prevent collateral circulation in subcutaneous tissue and muscle, an occluding rubber bandage was twisted around the root of the extremity and fixed around a pin inserted into the head of the humerus (axillary perfusion) or iliac crest (iliac perfusion). An inflating tourniquet was used in femoral or popliteal perfusions. Perfusion was performed during 90 min under mild hyperthermia (39-40\(^\circ\)C) and physiologically optimal conditions.\(^{16}\) At the start of perfusion, 3 mg (upper extremity) or 4 mg (lower extremity) TNF (Boehringer, Ingelheim, Germany) was injected as a bolus into the arterial line. Eighteen patients also received a dose of 0.2 mg INF (Boehringer, Ingelheim, Germany) subcutaneously 1 and 2 days before perfusion, followed by 0.2 mg INF injected into the arterial line at the start of perfusion. Melphalan (L-phenylalanine mustard, Glaxo-Wellcome, London, England) was administered 30 min later, as 10 mg/L extremity volume (leg) or 13 mg/L extremity volume (arm).\(^{17}\) The volume of the limb was determined before surgery by immersion.

All perfusions were performed with a bubble oxygenator roller pump and heat exchanger. The perfusate was oxygenated by a mixture of O\(_2\) and CO\(_2\) and consisted of 350 ml 5% dextran 40 in glucose 5% (Isodex, Pharmacia AB, Uppsala, Sweden), 500 ml blood (250 ml red blood cells, 250 ml plasma), 30 ml of 8.4% NaHCO\(_3\), 0.5 ml 5000 IU/ml heparin. The perfusions were flow regulated on the basis of the arterial and venous pressure measured at one end of the double lumen catheter used. After 90 min of perfusion, the limb was flushed with 2 L dextran 40 in glucose 5% (Isodex) and 500 ml blood (250 ml red blood cells, 250 ml plasma), catheters were removed, the circulation restored and the heparin antagonized with protamine chloride (Hoffman La Roche, Mijdrecht, the Netherlands). A lateral fasciotomy of the anterior compartment of the lower leg or arm was performed to prevent a compartment syndrome.\(^{18}\)
Leakage Measurement

Any leakage into the systemic circulation was continuously monitored with radioactive tracers. A small calibration dose of RISA (0.5 MBq) and a dose of radioactive Technetium-99m labeled human serum albumin (RTcSA; 10 MBq) were administered into the systemic circulation, after surgical isolation of the extremity was accomplished. The thyroid was saturated 1 day before the operation by oral administration of iodine (15 drops of Lugol solution twice daily). A 10 times higher dose of RISA (5 MBq) was injected into the perfusion circuit when perfusion was stable. The 364-keV gamma rays emerging from the RISA and the 140-keV gamma rays emerging from the RTcSA are measured with a NaI detector, which was placed in a flat field lead lined collimator that was mounted on an articulating mobile stand. This stand permits easy positioning of the detector, after it is covered with a sterile bag, above the heart of the patient. Careful attention is paid to ensure that the field of view of the detector did not cover parts of the HILP circuit. The detector signals generated by the photomultiplier tube were directed to an amplifier and then to a single-channel analyzer, allowing online data processing by a personal computer. The count rate of the 0.5-MBq RISA determined a baseline count level, corrected for room background. The 10-MBq RTcSA served to check the volume dilution caused by to fluid infusions or displacement of the NaI detector during the registration period. Leakage from the perfused limb to the systemic circulation resulted in an increase of the baseline count level. This increase, corrected for the blood volume ratio and the radioactivity ratio in both compartments, was a direct measure for the percentage of leakage and was continuously registered during the whole procedure. Stehlin was the first to describe the LF based on the following equation:

\[
\text{LF} = \frac{(c_{\text{systemic}} - c_{\text{baseline}})}{c_{\text{baseline}}} \cdot \frac{D_{\text{systemic}}}{D_{\text{perfusion}}} \cdot \frac{V_{\text{total}}}{V_{\text{systemic}}} \cdot 100\%
\]

where \(c_{\text{systemic}}\) is the systemic count rate observed during perfusion, \(c_{\text{baseline}}\) is the systemic count rate at the beginning of perfusion, \(D_{\text{systemic}}\) is the dose injected into the patient’s systemic circulation, and \(D_{\text{perfusion}}\) is the dose injected into the perfusion circuit \(V_{\text{total}}\) is the total blood volume (perfusion circuit + patient’s systemic circulation), \(V_{\text{systemic}}\) is the blood volume of the patient’s systemic circulation.\(^{11}\)

Blood sampling procedure and assays

A baseline blood sample from the patients systemic circulation was taken from an indwelling radial artery cannula before the start of the operation, and at 5, 30, 60,
and 89 min after the start of perfusion. Samples from the perfusion circuit were also taken at the same time intervals. After restoration of the circulation in the perfused limb, systemic samples were taken at 1, 5, 10, 30 and 60 min after removal of the arterial clamps, hourly thereafter for at least 8 hours and finally the next morning. Venous blood samples to study the hematological and metabolic profiles of urea nitrogen, creatinine, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gammaglutamyltranspeptidase, protein, cholesterol, lactic dehydrogenase (LDH) with its iso-enzymes, creatine phosphokinase and myoglobin were taken a day before perfusion, at the day of perfusion, and every day after perfusion until day 7. A final blood sample was taken one month after perfusion. Blood samples (3 ml) were collected in EDTA vacutainer tubes, and kept on melting ice during transport to a centrifuge. Samples were centrifuged for 10 min at 3000 rpm at 0°C and the separated plasma kept at –80°C until analysis.

TNF levels were determined by specific immunoradiometric assay (Medgenix Diagnostics, Soesterberg, the Netherlands). Samples were processed according to the guidelines of the manufacturer. IL-6 and CRP levels were measured by in-house sandwich ELISA’s as described previously19, by using commercial reagents for IL-6 (CLB, Amsterdam, the Netherlands; detection limit 10 ng/L) and for CRP (DAKO, Glostrup, Denmark; normal level <2.3 mg/L).

Assessment of tumor response
Responses were assessed by standardized World Health Organization criteria.20 Complete response (CR) was defined as the disappearance of all measurable disease in the limb for longer than 4 weeks, partial response (PR) as regression of the tumor size by >50% for longer than 4 weeks, and no change (NC) as regression of <50% of the tumor in the limb or progression of <25% for longer than 4 weeks. To analyze whether or not a high systemic leakage was of influence in the occurrence of distant metastasis subanalyses of this parameter in a group of patients with grade II and III soft tissue sarcomas was performed.

Statistical analysis
Values are expressed as mean ± SEM. Comparison between mean values of different groups was performed with the unpaired or in case of measuring the same variable in the same patient at different time points, with the paired Student’s t-test. Areas under the curve (AUC) were determined by the trapezoid rule. Survival curves were calculated according to the Kaplan Meier method and log rank test.21 Values of p ≤ .05 were considered to be statistically significant. Graph Pad Prism® version 2.0 for Windows (GraphPad, San Diego, CA) statistical software was used.
Results

Systemic leakage

For the 51 perfusions, the mean isotopically measured leakage was 2.9 % (95% confidence interval, 2.0 – 3.8%, range 0-15.5%). After 60 minutes of perfusion in the patient with the highest leakage (15.5%), it was noted that the rubber bandage twisted around the root of the extremity was ruptured. Since this was the cause of the high leakage and perfusion was not completed, the data from this patient are excluded from the remainder of the analyses. Systemic leakage was ≤ 2% in 28 perfusions (55%) and >2% in 23 perfusions (45%). In the latter group, 11 perfusions (22%) led to systemic leakage of >5%. In addition, analysis of different parameters between the group of patients with ≤ 2% leakage and the group of patients with >2% leakage, was made. Figure 1 shows the measured leakage at different perfusion levels. There was a significant difference between perfusions performed at the iliac and femoral levels compared with leakage values at the popliteal level, (p < 0.0001 and 0.0159 respectively). There was no leakage encountered in patients with axillary perfusions.

Perfusion circuit levels

At 5 minutes, mean TNF levels in the perfusion circuit were 6798 ± 528 ng/ml (Fig. 2). During perfusion, a significant drop in TNF levels in the perfusion circuit occurred with a significant lower concentration of TNF in the perfused limb in patients with >2% leakage at 30 (p = 0.0201), 60 (p = 0.0337) and 89 minutes (p = 0.002). The calculated mean AUC, indicating the exposure of the perfused limb to TNF, was 18.7% less in the >2% leakage group (p=0.0457). IL-6 levels in the perfusate, as one of the most important proinflammatory cytokines, progressively increased from 30 minutes until the end of the perfusion, reaching 4.2 ± 1.1 ng/ml in the ≤2% leakage group.
group and 11.7 ± 3.5 ng/ml in the >2% leakage group (p=0.0455). CRP levels in the perfusion circuit remained at the detection level, and no significant differences were observed between the leakage groups.

Fig. 2 Tumor necrosis factor (TNF) levels in the perfusion circuit (mean ± SEM). A significant decrease in TNF levels occurred with significant lower concentration of TNF in the perfused limb in patients with >2% leakage at 30 (p = 0.0201), 60 (p = 0.0337) and 89 minutes (p = 0.002). Mean area under the curve, indicating the exposure of the perfused limb to TNF, was 18.7% less in the >2% leakage group.

**Systemic levels**

Systemic TNF levels in patients with >2% leakage were already significantly higher at 5 min after TNF injection compared with the group of patients with ≤2% leakage. Peak systemic TNF values of 116.5 ± 28.9 ng/ml were reached in the >2% leakage group at the end of perfusion, compared with 11.8 ± 3.4 ng/ml in the ≤2% leakage group (p < 0.0001) (Fig. 3). The calculated mean systemic AUC, indicating the exposure of the patient to TNF, was 18.1 times higher in the >2% leakage group (p<0.0001). Ten minutes after release of the tourniquet we observed a significant systemic peak level of TNF in the ≤2% group possibly caused by the TNF still present in the perfused limb after the washout procedure (p=0.026). To calculate the correlation between maximum systemic TNF levels and the maximum monitored leakage using RISA measured during perfusion, Pearson’s correlation (two-tailed) was used. Figure 4 illustrates the observed correlation with r = 0.7114 and p < 0.0001. A strong correlation was also found between the maximal observed leakage and maximum IL-6 concentration measured in the postoperative period (r = 0.7737, P<0.0001).

IL-6 levels appeared in the systemic circulation 30 minutes after the start of the perfusion and maximal levels were reached 2 hours after HILP (19.5 ± 5.8 ng/ml ≤2% leakage versus 77.7 ± 20.8 ng/ml >2% leakage; p=0.0089). The AUC of IL-6 was 4.7 times higher in the >2% leakage group compared with ≤2% leakage group (p<0.0243).
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Fig. 3 Tumor necrosis factor (TNF) levels in the systemic circulation of the patient (mean ± SEM). A significant difference was found between the >2% leakage group and the ≤2% leakage group is starting 5 min after TNF injection until the second postoperative day ($p<0.05$). The mean systemic area under the curve, indicating the exposure of the patient to TNF, was 18-times higher in the >2% leakage group.

Fig. 4 Pearson’s correlation (two-tailed) between maximal systemic TNF levels measured during perfusion and maximal monitored leakage using RISA ($r = 0.7114$ and $P<0.0001$)

CRP started to increase 6 hours after HILP and reached its maximal value 2 days after perfusion (185.8 ± 25.5 mg/L ≤2% leakage versus 226.7 ± 32.7 mg/L, >2% leakage; not significant). The AUC of CRP between both groups however was not significantly different.

**Hematological and metabolic parameters**

Leukocyte counts increased from 7.7 ± 0.3x10⁹/L to 13.0 ± 0.6x10⁹/L one day after perfusion. Five, 6, and 7 days after perfusion a significant difference between the two leakage groups was observed (Fig. 5). Platelet counts decreased from 303.6 ± 13.4x10⁹/L before perfusion to 124.3 ± 10.7x10⁹/L 4 days after perfusion.
The low platelet levels persisted longer in the >2% leakage group. Kidney function was well preserved in all patients, although urea nitrogen and creatinine levels in the >2% leakage group were significantly higher during the first 5 days after perfusion; these levels however remained within normal limits. Liver function tests showed an increase in bilirubin values from $10.5 \pm 0.9 \mu\text{mol/L}$ to $44.8 \pm 11.3 \mu\text{mol/L}$, 4 days after perfusion in the >2% leakage group, with significant differences compared with the ≤2% leakage group (Fig. 6). Figure 6 illustrates the decrease in protein levels and cholesterol levels after perfusion, with significant differences between both leakage groups. Alkaline phosphatase increased from $86.1 \pm 6.5 \text{U/L}$ to $159.4 \pm 32.8 \text{U/L}$, aspartate aminotransferase increased from $22.8 \pm 1.5 \text{U/L}$ to a maximum of $62.1 \pm 13.4 \text{U/L}$ on the fifth day after perfusion, alanine aminotransferase increased from $21.9 \pm 2.6 \text{U/L}$ to a maximum of $80.3 \pm 11.6 \text{U/L}$ on the sixth day after perfusion, and gammaglutamyltransferase increased from $37.7 \pm 8.7 \text{U/L}$ to a maximum of $120.1 \pm 18.6 \text{U/L}$ on the sixth day after perfusion. LDH increased from $224.8 \pm 9.0 \text{U/L}$ to a maximum of $417.3 \pm 19.1 \text{U/L}$ on the second day after perfusion. LDH iso-enzymes 1 and 2 showed a decrease, whereas LDH iso-enzymes 4 and 5 increased one day after perfusion. LDH iso-enzyme 3 remained at the same level. Creatine phosphokinase levels increased from $28.3 \pm 2.4 \text{U/L}$ to a maximum of $496.4 \pm 197.6 \text{U/L}$ on

![Fig. 5](image-url) White blood cell count (WBC) and platelets (PLT) levels from before perfusion to 30 days after perfusion (mean ± SEM). * indicates a significant difference between both leakage groups ($p < 0.05$)
the second day after perfusion. Myoglobin levels increased from $30.2 \pm 2.4 \, \mu g/L$ to a maximum of $422.8 \pm 99.7 \, \mu g/L$ one day after perfusion. None of these variables showed a significant difference between both leakage groups.

**Tumor Response**

In the $\leq 2\%$ leakage group, 14 patients showed a CR and the same number of patients a PR. In the $>2\%$ leakage group, 11 patients showed a CR, 10 patients a PR and 2 patients had NC. No significant differences in response to TNF HILP were found between the groups. No significant difference was observed in the occurrence of distant metastasis or survival in the subanalyses of a group of patients with grade II or III soft tissue sarcoma and $>2\%$ leakage (*Fig. 7*).
Discussion

The purpose of continuous leakage monitoring with RISA during HILP is to indicate the amount of chemotherapeutic agent that is leaking from the perfusion circuit into the patient’s systemic circulation. When leakage occurs, measures to reduce leakage should be available. During perfusion there is a dynamic balance between two pressure compartments: the patient’s systemic vasculature and the isolated circuit. The pressure of the former compartment can be influenced by adjusting the systemic blood pressure, whereas that of the latter can be affected by alterations in the extracorporeal flow rate. Thus, to decrease leakage, the anesthesiologist can increase the patient’s blood pressure or the surgeon can reduce the flow rate in the heart-lung machine. Different methods for measurement of leakage have been used. The previously described method with RISA is the most frequently used; however, a MEDLINE search to find any articles calculating the correlation between systemic melphalan levels and leakage in case of HILP with melphalan only, produced no results. The first report on TNF levels after HILP with TNF, from Gérain et al. in 1992, demonstrated no significant correlation between leakage and cytokine levels at any time, raising questions about the value of the leakage measurement procedure.

The aim of this study was to investigate whether or not the RISA leakage measurements during HILP with TNF used in the Groningen University Hospital are accurate in predicting systemic TNF levels. We observed a good correlation between maximal systemic TNF levels and the maximum monitored leakage ($r = 0.7114; p < 0.0001$). We were surprised to find that the correlation between maximal leakage and maximal IL-6 concentration measured in the postoperative period was higher than the correlation between maximal leakage and maximal TNF levels ($r = 0.7737$ versus $r = 0.7114$). IL-6 levels occurred in response to TNF, with a high correlation between maximal levels of both cytokines ($r = 8097$). Stam et al. also found a strict correlation between the degree of leakage estimated by isotope monitoring and the
measured maximal systemic TNF levels in the same treatment setting \( (r = 0.7886, p = 0.0067) \); calculation based on their data. They also found a sharper relation between systemic IL-6 curves and duration of exposure to high TNF levels in patients with high leakage compared with a group of patients with no leakage. A significant difference in leakage was found between the iliac/femoral perfusion levels and popliteal perfusion level. This corresponds with the study of Klaase et al., who assessed six variables for their influence on systemic leakage. The level of isolation and the diameter of the venous cannula emerged as significant factors. In our study we could not find a significant role for the diameter of the venous cannula (data not shown). The importance of the perfusion level could be partly explained by the different type of isolation technique used, namely, a rubber band tourniquet at the iliacal level versus an inflatable pressure regulated band at the popliteal level.

In the analysis of our data, we distinguished two leakage groups, with a cutoff point at 2%. Two percent represents approximately the measurement fault of the RISA procedure. TNF levels in the perfusion circuit were about 7000 ng/ml, approximately 50-times higher than peak systemic levels. A significantly lower concentration of TNF in the perfused limb in patients with >2% leakage was demonstrated resulting in a decreased AUC, indicating an 18.7% lower exposure of the perfused limb to TNF in the >2% leakage group. This decrease in TNF exposure, however, did not result in a significant reduction of tumor response between the groups. This result supports the initiation of TNF dose reduction studies. Thom et al. observed the same decreased TNF perfusion circuit levels in patients with ≥1% leakage.

The Rotterdam perfusion group did not demonstrate a significant difference in perfusion circuit TNF levels between a high and low-leakage group, possibly because of a limited number of samples available. TNF levels in the systemic circulation of the patients were approximately 100 ng/ml in the >2% leakage group at the end of perfusion, compared with 10 ng/ml in the ≤2% leakage group. In patients with ≤2% leakage, systemic TNF exposure was 18.1 times less as calculated by the AUC. On the basis of the hypothesis that micro metastatic disease is attacked by the leaked TNF and melphalan, a higher systemic exposure of TNF could have its effect on the occurrence of distant metastasis during follow-up. However, subanalysis of the occurrence of distant metastasis or survival in a group of patients with grade II or III soft tissue sarcomas did not reveal this phenomenon. IL-6, as one of the most important proinflammatory cytokines, appeared in the systemic circulation 30 minutes after the start of the perfusion with maximum levels reached 2 hours after HILP. CRP levels started to increase 6 hours after HILP and reached its maximum 2 days after perfusion. A three wave pattern was seen; the first wave caused by the systemically leaked TNF that generated a second wave of
IL-6 some hours after perfusion, followed by a third wave of CRP that lasts for several days.

TNF leakage was associated with a decrease in leucocyte and platelet count, with significantly lower values in the >2% leakage group. Representing cytolytic liver toxicity, a significantly hyperbilirubinemia, hypocholesterolemia and proteinemia was observed in the >2% leakage group. A increase in the activity of the fraction of LDH iso-enzymes 4 and 5 after perfusion was partly related to hepatotoxicity and partly to muscle damage. No significant difference between both leakage groups was found for creatine phosphokinase levels or myoglobin levels although both parameters showed a significant rise after HILP. The same results were obtained by Sorkin et al. who diminished TNF leakage after flow rate reduction during TNF HILP. Analysis of our own flow data in relation to systemic leakage revealed a weak negative correlation of $r = -0.2910$ with $p = 0.0448$ with a mean flow of $455 \pm 172$ ml/min in our perfusions.

Like others, we also found a significant systemic TNF peak in patients with low leakage after restoration of the circulation of the perfused limb. Despite a washout procedure with 2 L of Isodex, TNF in the limb reaches the systemic circulation. A corresponding rise in RISA was also observed. Therefore, today a more extensive washout with 6 L and massage of the perfused limb is recommended in TNF perfusions to reduce TNF release.

In a previous study we described the clinical features of HILP with TNF characterized by a short- lived sepsis-like syndrome. This best called SIRS, was seen in all patients and accompanied by fever, increase in cardiac output, a decrease in systemic vascular resistance, and the need for fluid resuscitation and inotropes. Perfusion with melphalan as the sole perfusion agent did not trigger these effects. Detailed analysis showed positive correlations between maximum TNF concentrations and systemic vascular resistance and cardiac index. The National Cancer Institute perfusion group demonstrated the relation between the vascular response with the need for vasopressor support and systemic TNF levels in patients with TNF leakage as well. Lejeune also demonstrated severe toxicity in patients with leaks of >5%. Vrouwenraets et al. reported an absence of severe systemic toxicity of TNF in patients without systemic leakage. Stam et al. observed only a mild postoperative toxicity in the event of significant leakage during perfusion. This was easily managed on the intensive care unit with fluid substitution and, in some cases, vasopressors. On the basis of their data, they rightly plead for renewed study of the potential use of TNF systemically. Currently, SIRS is only seldom seen since the majority of the institutions performing HILP with TNF and melphalan are experienced and are using a more extensive washout procedure. One could ask oneself if leakage measurements during
HILP are still worthwhile when side effects of TNF leakage are so easily dealt with. In this study we demonstrated a good correlation between RISA leakage measurement and TNF exposure during and after HILP with TNF and melphalan. RISA leakage measurement serves as a good guide for the effectiveness of isolation during perfusion. If leakage exceeds the 2% limit during perfusion, less exposure of the tumor bearing limb to TNF, an increased exposure of the patient’s systemic circulation to TNF, and more systemic side effects can be expected. Because leakage >2% did not influence the tumor response, further dose-reduction studies of TNF in the HILP setting are warranted.
References

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