ORIGINAL ARTICLE

# Isolated Limb Perfusion

## *Distinct Tourniquet and Tumor Necrosis Factor Effects on the Early Hemodynamic Response*

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**Hypothesis:** Recent evidence indicates that tumor response rates after isolated limb perfusion (ILP) are improved when tumor necrosis factor (TNF) is added to the locoregional perfusion of high doses of chemotherapy. Other factors, related to the patient or the ILP procedure, may interfere with the specific role of TNF in the early hemodynamic response after ILP with TNF and high-dose chemotherapy.

**Design:** Case-control study.

**Setting:** Tertiary care university hospital.

**Patients:** Thirty-eight patients with a locoregionally advanced tumor of a limb treated by ILP with TNF and high-dose chemotherapy (TNF group) were compared with 31 similar patients treated by ILP with high-dose chemotherapy alone (non–TNF group).

**Interventions:** Swan-Ganz catheter hemodynamic recordings, patients' treatment data collection, and TNF and interleukin 6 plasma level measurements at regular intervals during the first 36 hours following ILP.

**Main Outcome Measures:** Hemodynamic profile and total fluid and catecholamine administration.

**Results:** In the TNF group, significant changes were observed ($P<.006$): the mean arterial pressure and the systemic vascular resistance index decreased, and the temperature, heart rate, and cardiac index increased. These hemodynamic alterations started when the ILP tourniquet was released (ie, when or shortly after the systemic TNF levels were the highest). The minimal mean arterial pressure, the minimal systemic vascular resistance index, the maximal cardiac index, the intensive care unit stay, and the interleukin 6 maximal systemic levels were significantly ($P<.001$ for all) correlated to the $\log_{10}$ of the systemic TNF level. In the non–TNF group, only a brief decrease in the blood pressure following tourniquet release and an increase in the temperature and in the heart rate were statistically significant ($P<.006$). Despite significantly more fluid and catecholamine administration in the TNF group, the mean arterial pressure and the systemic vascular resistance index were significantly ($P<.001$) lower than in the non–TNF group.

**Conclusions:** Release of the tourniquet induces a blood pressure decrease that lasts less than 1 hour in the absence of TNF and that is distinct from the septic shock–like hemodynamic profile following TNF administration. The systemic TNF levels are correlated to this hemodynamic response, which can be observed even at low TNF levels.

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TUMOR NECROSIS factor (TNF) is a trimeric cytokine secreted by several cell types, including macrophages. It was identified in 1975 as the factor causing hemorrhagic necrosis of subcutaneous tumors in mice injected with endotoxin.[1] After human TNF recombination, the enthusiastically expected phase 1 trials of systemic administration turned out to be disappointing. Practically no antitumoral effect was observed, because the maximal tolerated dose was limited to insufficient levels by significant systemic toxicity, mainly fever, chills, malaise, anorexia, thrombocytopenia, and hypotension.[2-5] Pessimism increased further because in the same years TNF was shown to play a major role in the inflammatory cascade leading to septic shock.

Isolated limb perfusion (ILP) is a locoregional cancer treatment that allows high doses of antitumoral drugs to be perfused through the vascular bed of a limb while avoiding toxic systemic concentrations. It was first described in 1958,[6] but gained wide acceptance only after Lienard et al[7] reported 90% complete remission rates for advanced malignant melanoma and soft tissue sarcoma following hyperthermic ILP with high doses of melphalan combined with TNF and interferon gamma-1b. This significant antitumoral effect is caused by destruction of the



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**Table 1. Patients' Clinical Characteristics***

| Characteristic | TNF Group (n = 38) | Non–TNF Group (n = 31) |
|---|---|---|
| Treatment | | |
| Melphalan | | |
| Alone | 0 | 28 |
| Plus TNF | 11 | 0 |
| Plus TNF and interferon gamma-1b | 27 | 0 |
| Fotemustine | 0 | 3 |
| Male-female ratio | 24:14 | 18:13 |
| Age, mean (range), y | 54.6 (19-79) | 58.7 (30-87) |
| Indication for ILP | | |
| Malignant melanoma | 30 | 29 |
| Soft tissue sarcoma | 5 | 1 |
| Squamous cell carcinoma | 2 | 0 |
| Merkel cell carcinoma | 0 | 1 |
| PNET | 1 | 0 |
| Limb affected | | |
| Lower | 30 | 29 |
| Upper | 8 | 2 |
| ASA score ≥3 | 5 | 5 |
| History of coronary disease, cardiac failure, or atrial fibrillation | 6 | 3 |

Abbreviations: ASA, American Society of Anesthesiologists; ILP, isolated limb perfusion; PNET, peripheral neuroectodermal tumor; TNF, tumor necrosis factor.

*Data are given as number of patients in each group unless otherwise indicated. Patients in the TNF group were treated with ILP and high-dose chemotherapy, with TNF; and those in the non–TNF group, with ILP and high-dose chemotherapy, without TNF. The only significant difference between the 2 groups was in the number of patients with an upper limb affected ($P = .07$).

tumor at 2 levels: in addition to the cytotoxic effect of melphalan, TNF selectively induces apoptosis of endothelial cells of the tumor vessels, leading to the disruption of the tumor vasculature and to coagulative necrosis.[8] However, leakage from the ILP circuit can lead to high levels of TNF in the systemic circulation. A septic shock–like syndrome with hypotension and low systemic vascular resistances after TNF administration and ILP has indeed been described,[9-12] although plasma TNF levels up to 10 000 times higher than those observed with lethal septic shock were not fatal. However, the hemodynamic response to TNF was variable and the role of TNF vs surgical and anesthesia variables was not determined. This prompted us to investigate the role of TNF in the early hemodynamic response to ILP. We collected retrospectively the data of 38 patients who underwent ILP with TNF and chemotherapy and compared them with those of 31 patients treated with ILP and chemotherapy only.

## METHODS

### PATIENTS

Between February 5, 1992, and September 7, 1994, 38 consecutive patients underwent hyperthermic ILP with TNF and melphalan, with or without interferon gamma-1b (TNF group). They were compared with 31 patients who underwent ILP without TNF from May 26, 1993, to October 17, 2000, with either melphalan (n=28) or fotemustine (n=3) (non–TNF group). All patients were operated on at the Centre Hospitalier Universitaire Vaudois, by the same team of surgeons (F.J.L., F.M., and D.L.) and anesthetists (P.-G.C.), except 4 patients in the non–TNF group who were treated at the Hospital São Paulo by one of us (F.B.). The indications for ILP were malignant melanoma, soft tissue sarcoma, squamous cell carcinoma, Merkel cell carcinoma, and peripheral neuroectodermal tumor, always at a locoregionally advanced stage. The patients' main clinical characteristics are shown in **Table 1**. All patients treated with TNF, melphalan, or fotemustine were included in phase 1 and 2 studies approved by the local ethics committee; most studies[13-17] were published.

### ISOLATED LIMB PERFUSION

The details of the technique have been published before.[7,13] In brief, the major artery and vein of the diseased limb (iliac, femoral, or axillary) are clamped and cannulated. Isolation of the extracorporeal circuit is obtained by ligation of the collateral vessels and application of a tourniquet at the root of the limb. After ILP is started, the temperature of the limb is increased and maintained between 39°C and 40°C using a warming blanket and heated perfusions. When stable perfusion conditions are reached, leakage from the limb into the systemic circulation is assessed by the technetium Tc 99m albumin radiolabeled human serum method.[18,19] When the leakage rate is nil or minimal, TNF is injected as a bolus into the circuit. Thirty minutes later, melphalan or fotemustine is added and circulates for another 60 minutes. The total drug perfusion time is, therefore, 90 minutes in the TNF group and 60 minutes in the non–TNF group. At the end of the perfusion, the limb is rinsed for at least 10 minutes with 4 to 5 L of a colloid solution before the tourniquet is released. The vein and then the artery are decannulated and repaired.

### ANESTHESIA

The induction of anesthesia and the use of muscular relaxants were similar in both groups. In all the TNF group patients, anesthesia was maintained mainly by benzodiazepines and fentanyl citrate. Isoflurane was administered to only 12 (32%) of these patients to avoid hypotension; nitrous oxide was used in 7 (18%) of the patients. On the contrary, in the non–TNF group, isoflurane was used in 30 (97%) of the patients and nitrous oxide in 19 (61%) of the patients; only 1 (3%) of the patients received long-acting benzodiazepines ($P<.01$). Dopamine hydrochloride, 3 to 5 µg/kg per minute, was administered prophylactically from the beginning to all but 1 of the patients in the TNF group. In the non–TNF group, intraoperative dopamine was given only as clinically indicated (to 9 [29%] of the 31 patients). Intravenous indomethacin was administered to the TNF group patients only (50 mg over 4 hours, followed by 200 mg over the next 20 hours), to decrease systemic inflammatory adverse effects.

### DRUGS

The TNF group patients received this cytokine as a 4- or 3-mg bolus for a lower or an upper limb, respectively. For safety reasons (poor leakage control or hypotension), one patient received 2 mg initially and 1 mg 30 minutes later, instead of a single 4-mg bolus. A second patient received 1 mg initially and 1 mg 30 minutes later instead of the planned 4-mg bolus. Finally, a third patient received 2 mg initially and 1 mg 30 minutes later instead of a 3-mg bolus.

Twenty-seven patients in the TNF group also received 0.2 mg (or $1.5\times10^6$ U) of interferon gamma-1b subcutaneously for 2 days before ILP, and another dose was injected in the per-



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fusion circuit at the beginning of the procedure. Melphalan (Alkeran; Glaxo Wellcome Inc, Uxbridge, England) was given 30 minutes after TNF as a bolus of 10 or 13 mg/L of lower or upper limb volume, respectively (range, 32-115 mg). Fotemustine (Muphoran; Servier, Nevilly-sur-Seine CEDEX, France) was administered as melphalan, at a dose of 25 mg/L of limb volume.

### HEMODYNAMIC DATA

A 7F Swan-Ganz catheter (Baxter Healthcare Corp, Deerfield, Ill) was positioned after the induction of anesthesia in all the TNF group patients. The temperature, heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), mean pulmonary arterial pressure, pulmonary capillary wedge pressure (PCWP), cardiac index (CI), and stroke volume index (SVI) were recorded at least once during 8 predetermined periods before, during, and after ILP. These periods were defined as follows: (1) during anesthesia, before ILP; (2) during ILP, before drug perfusion; (3) during ILP with drug perfusion, before tourniquet release; (4) after tourniquet release and up to 3 hours after drug perfusion; (5) 3 to 6 hours after the initiation of drug perfusion; (6) 6 to 12 hours after the initiation of drug perfusion; (7) 12 to 24 hours after the initiation of drug perfusion; and (8) 24 to 36 hours after the initiation of drug perfusion. Cardiac output was measured in triplicate by the thermodilution method, injecting 10 mL of 5% glucose at room temperature.[20] Whenever more than 1 measurement was performed during a given period, the arithmetic mean was calculated. The systemic vascular resistance index (SVRI) was calculated as follows:

$$SVRI = [(MAP - CVP) \times 80]/CI$$

The pulmonary vascular resistance index (PVRI) was calculated as follows:

$$PVRI = [(MAPP - PCWP) \times 80]/CI$$

In the non–TNF group, 12 patients had Swan-Ganz catheter measurements (December 2, 1998–October 17, 2000); only basic data (blood pressure, HR, and temperature) were recorded in the other 19 patients.

To define more precisely the tourniquet release effect, the HR and the systolic and diastolic pressures were recorded in all patients 10 minutes before and then 5, 30, and 60 minutes after the release. From these recordings, the MAP was calculated as follows:

$$MAP = \text{Diastolic Pressure} + [(\text{Systolic Pressure} - \text{Diastolic Pressure})/3]$$

### CYTOKINE LEVELS

All TNF group patients underwent measurement of systemic levels of TNF and interleukin 6 (IL-6) during and up to 36 hours after the initiation of ILP. The TNF level was measured using the WEHI 164 clone 13 cell line.[21] A standard curve was obtained using TNF from the same batch as that given to the patients. The lower limit of detection was 0.5 pg/mL.

The level of IL-6 was measured using IL-6–dependent mouse-mouse hybridoma cells (7TD1). Interleukin 6 concentrations were expressed in units per milliliter, with 1 U being the dose of IL-6 inducing a 50% proliferation.

### STATISTICAL ANALYSES

Data were analyzed using a statistical software program (Statistical Product and Service Solutions, version 8.0; SPSS Inc, Chicago, Ill). Patients' characteristics (Table 1) and the percentage of patients treated with catecholamines (**Table 2**) from the TNF group and the non–TNF group were compared using the Pearson $\chi^2$ test. Patients' treatment variables (**Table 3**) were compared between groups using the *t* test for comparison of means. Hemodynamic measurements are given as mean ± 95% confidence interval (**Figures 1**, **2**, **3**, and **4**). A 1-way analysis of variance for repeated measurements was used to compare the values at different time points in a given patient group, with Tukey and Bonferroni corrections for 8 repeated measures (periods 1-8) or for 4 repeated measures (tourniquet recordings). To compare values between groups at a given time point, the 2-tailed *t* test was used. Correlations to TNF (**Table 4**) were calculated by linear regression analysis with logarithmic transformations when necessary to ensure a normal distribution.

**Table 2. Patients Treated With Catecholamines***

| Catecholamine | TNF Group (n = 38) | Non–TNF Group (n = 31) | *P* Value |
|---|---|---|---|
| Dopamine hydrochloride† | 37 (97) | 11 (35) | <.001 |
| Norepinephrine bitartrate‡ | 28 (74) | 2 (6) | <.001 |
| Dobutamine hydrochloride§ | 14 (37) | 2 (6) | .01 |
| Epinephrine | 3 (8) | 0 | . . . |

Abbreviation: TNF, tumor necrosis factor.
*Data are given as number (percentage) of patients unless otherwise indicated. The TNF group and the non–TNF group are described in the second footnote to Table 1.
†Those treated with 400 µg/min or more included 11 (29%) of the patients in the TNF group and 3 (10%) of the patients in the non–TNF group.
‡Those treated with 10 µg/min or more included 18 (47%) of the patients in the TNF group and 0 patients in the non–TNF group.
§Those treated with 300 µg/min or more included 12 (32%) of the patients in the TNF group and 1 (3%) of the patients in the non–TNF group.

**Table 3. Patient Treatment Variables***

| Variable | TNF Group | Non–TNF Group | *P* Value |
|---|---|---|---|
| RBCs transfused intraoperatively, U | 3.9 ± 1.1 | 1.2 ± 0.5 | <.001 |
| Postoperative hemoglobin level, g/dL | 10.2 ± 0.5 | 9.4 ± 1.0 | .11 |
| Fluid balance, L | | | |
| At 24 h | 8.5 ± 1.4 | 5.7 ± 0.7 | .005 |
| At 36 h | 8.6 ± 1.5 | 5.5 ± 0.9 | .002 |
| Operation duration, min | 314 ± 14 | 277 ± 20 | .001 |
| ICU stay, h | 75.9 ± 13.3 | † | . . . |
| Tracheal intubation time, h | 37.9 ± 7.7 | ‡ | . . . |

Abbreviations: ICU, intensive care unit; RBC, red blood cell; TNF, tumor necrosis factor.
*Data are given as mean ± 95% confidence interval. The TNF group and the non–TNF group are described in the second footnote to Table 1.
†Only 2 patients needed an ICU stay.
‡All patients were extubated in the operating room.

## RESULTS

### HEMODYNAMIC DATA

The evolution of the mean temperature, HR, MAP, CVP, pulmonary arterial pressure, PCWP, CI, SVRI, SVI, and PVRI in the TNF group and the non–TNF group is presented in Figures 1 through 4.

The temperature differed significantly between the 2 groups 3 to 24 hours after ILP: fever was observed in the TNF group only 6 to 12 hours after ILP (maximum mean temperature, 38.1°C).

The mean HR in the TNF group patients increased to a maximum of 120 beats/min (166% of baseline) 3 to 6 hours post-ILP; it decreased thereafter, but tachycar-



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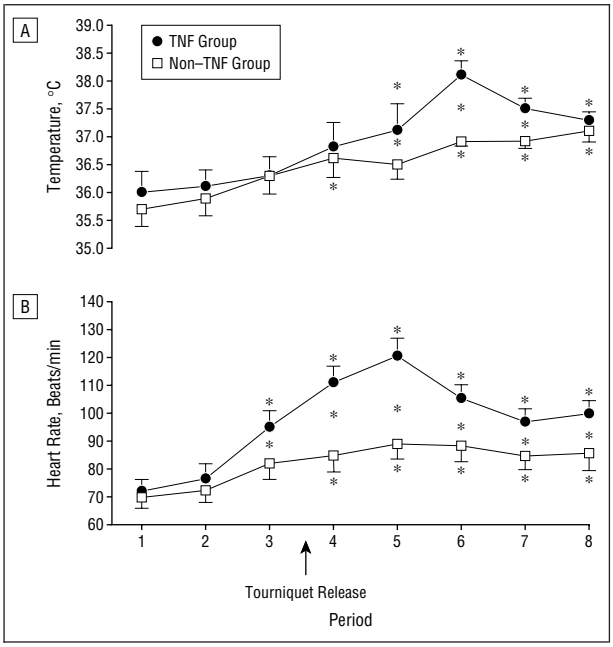


**Figure 1.** Changes in temperature (A) and heart rate (B) during periods 1 through 8 (defined in the "Hemodynamic Data" subsection of the "Methods" section). Data are given as mean±95% confidence interval. The TNF group indicates patients treated with isolated limb perfusion (ILP) and high-dose chemotherapy, with tumor necrosis factor (TNF); non–TNF group, patients treated with ILP and high-dose chemotherapy, without TNF; an asterisk between the curves, a significant ($P<.05$) difference between the TNF group and the non–TNF group; and an asterisk above or below the curves, a significant ($P<.006$) difference of the corresponding reading compared with baseline (period 1).

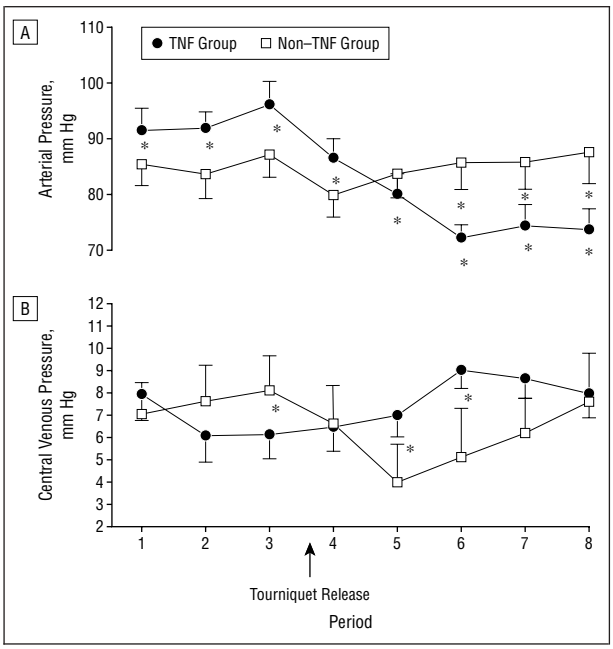


**Figure 2.** Changes in arterial pressure (A) and central venous pressure (B) during periods 1 through 8 (defined in the "Hemodynamic Data" subsection of the "Methods" section). Data are given as mean±95% confidence interval. A description of the groups and an explanation of the abbreviations and symbols are given in the legend to Figure 1.

dia persisted for at least 36 hours. The non–TNF group also showed a significant, but milder, increase in the mean HR, reaching 89 beats/min (127% of baseline).

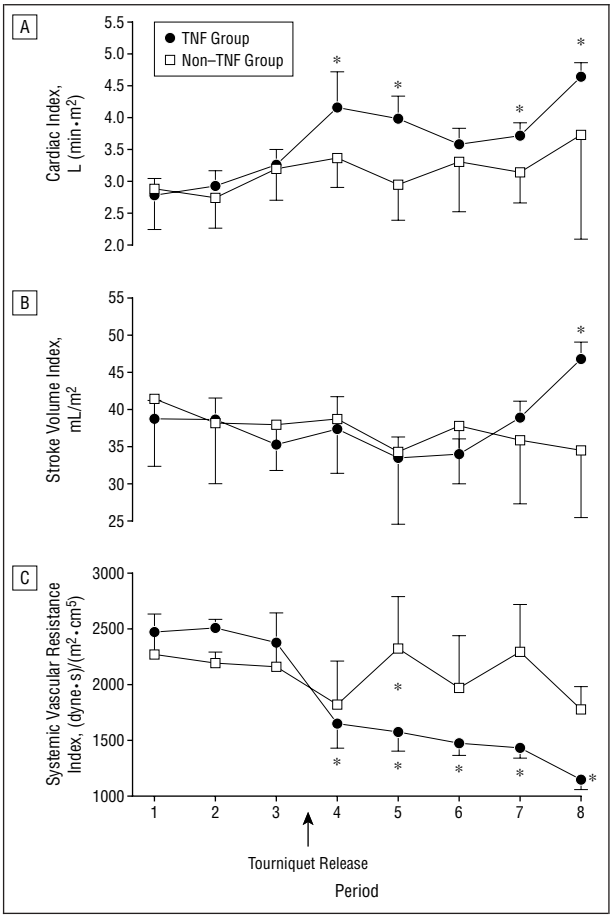


**Figure 3.** Changes in the cardiac index (A), the stroke volume index (B), and the systemic vascular resistance index (C) during periods 1 through 8 (defined in the "Hemodynamic Data" subsection of the "Methods" section). Data are given as mean±95% confidence interval. A description of the groups and an explanation of the abbreviations and symbols are given in the legend to Figure 1.

The MAP was significantly higher during anesthesia (periods 1-4) in the TNF group ($P=.03$). This may be explained by the more frequent use of isoflurane in the non–TNF group and the prophylactic early intraoperative administration of dopamine in the TNF group patients.

In the TNF group, the MAP began to decrease after tourniquet release and reached a nadir of 72.4 mm Hg (79% of baseline) at 6 to 12 hours after ILP. It remained low for at least 36 hours (range of individual minimal recordings, 53-59 mm Hg). It was significantly ($P<.001$) lower in the TNF group than in the non–TNF group at 6 to 36 hours after ILP. In the non–TNF group, no significant ($P=.29$) variation of the MAP was noticed, as recorded and averaged during periods 1 to 8, although the MAP after tourniquet release during period 4 decreased from 87.2±4.1 to 79.9±3.9 mm Hg ($P=.60$).

The CVP in the TNF group was significantly higher during period 6 than during ILP (periods 2 and 3). This was not the case in the non–TNF group.

In the TNF group, the CI (HR×SVI) increased significantly ($P<.001$) after tourniquet release, explained by an HR increase despite a decrease of the SVI. The highest recording was obtained 24 to 36 hours after ILP, because of a marked increase of the SVI. These changes did not oc-



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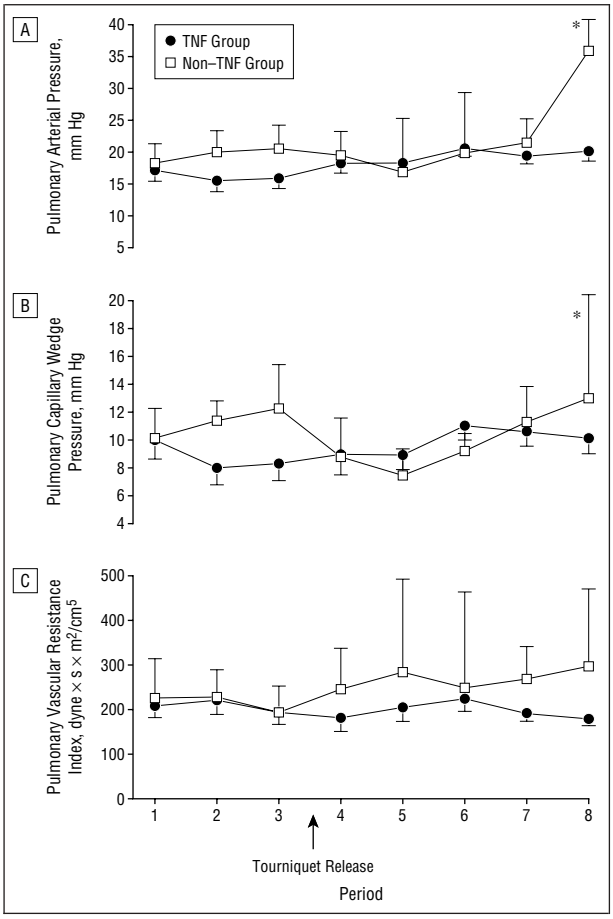


**Figure 4.** Changes in the pulmonary arterial pressure (A), the pulmonary capillary wedge pressure (B), and the pulmonary vascular resistance index (C) during periods 1 through 8 (defined in the "Hemodynamic Data" subsection of the "Methods" section). Data are given as mean ± 95% confidence interval. The TNF group indicates patients treated with isolated limb perfusion (ILP) and high-dose chemotherapy, with tumor necrosis factor (TNF); non–TNF group, patients treated with ILP and high-dose chemotherapy, without TNF; and an asterisk, the 95% confidence interval exceeds the graph scale.

cur in the non–TNF group, but the limited number of recordings does not allow firm conclusions to be drawn.

The SVRI decreased sharply (–31%) in the TNF group after tourniquet release ($P<.001$). Thereafter, the readings were also below normal and significantly ($P=.002$) lower than in the non–TNF group. The lowest figures were obtained 24 to 36 hours after ILP. In the non–TNF group, the SVRI tended to decrease (–16%) on tourniquet release ($P=.76$) and was variable thereafter. Again, the limited number of measurements does not allow definitive conclusions.

The mean pulmonary arterial pressure and the PCWP varied little and remained within the normal range. In the TNF group, they tended to decrease during perfusion and were slightly, but not significantly ($P=.13$), higher than baseline readings 24 to 36 hours after ILP. During ILP, the PCWP was slightly, but not significantly, higher in the non–TNF group. The PVRI in the TNF group did not vary significantly ($P=.15$) throughout the observation time.

The effects of tourniquet release on HR and MAP in the non–TNF group (at 10 minutes before and 5, 30, and 60 minutes after tourniquet release) are shown in **Figure 5**:

**Table 4. Correlations to the $Log_{10}$ of the Peak Systemic TNF Level**

| Variable | *r* | *P* Value |
|---|---|---|
| Log of the IL-6 max | 0.79 | <.001 |
| Stay at the ICU (in hours) | 0.68 | <.001 |
| MAP | | |
| $Min_{3-8}$ | 0.59 | <.001 |
| Decrease* | 0.38 | .02 |
| CI | | |
| $Max_{3-8}$ | 0.55 | <.001 |
| Increase† | 0.38 | .03 |
| SVRI | | |
| $Min_{3-8}$ | 0.53 | <.001 |
| Decrease‡ | 0.28 | .12 |
| Log of total norepinephrine bitartrate given in 36 h | 0.30 | .14 |
| Log of liters of positive fluid balance at 36 h | 0.23 | .17 |
| Temperature $max_{3-8}$ | . . . | . . . |
| Heart rate $max_{3-8}$ | . . . | . . . |

Abbreviations: CI, cardiac index; ICU, intensive care unit; IL-6, interleukin 6; MAP, mean arterial pressure; max, maximum; $max_{3-8}$, maximum during periods 3 through 8; $min_{3-8}$, minimum during periods 3 through 8; SVRI, systemic vascular resistance index (periods are defined in the "Hemodynamic Data" subsection of the "Methods" section); TNF, tumor necrosis factor.

*Calculated as follows: MAP during period 1 minus MAP $min_{3-8}$.

†Calculated as follows: CI $max_{3-8}$ minus CI during period 1.

‡Calculated as follows: SVRI during period 1 minus SVRI $min_{3-8}$.

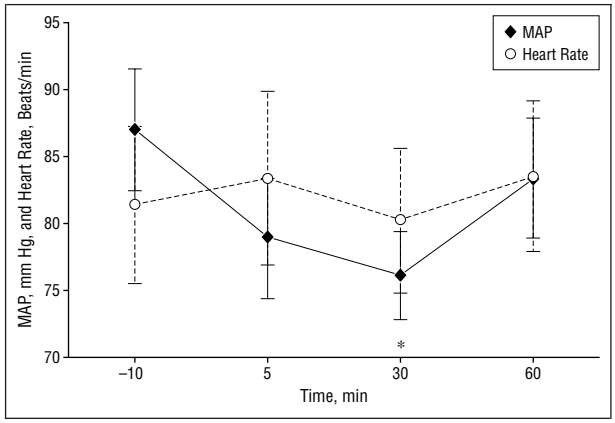


**Figure 5.** Tourniquet effects on mean arterial pressure (MAP) and heart rate in the patients treated with isolated limb perfusion and high-dose chemotherapy, without tumor necrosis factor. Data are given as mean ± 95% confidence interval. The asterisk indicates a significant difference ($P<.01$) between the corresponding reading and the –10-minute value.

the MAP decreased by 10% ($P=.27$) and by 13% ($P<.01$) at 5 and 30 minutes, respectively, returning to baseline at 60 minutes (–4%) ($P=.76$). In the TNF group, compared with the readings at 10 minutes before release, the MAP decreased by 8% ($P=.02$) and by 11% ($P<.01$) at 5 and 30 minutes, respectively. Conversely to the non–TNF group, it remained low at 60 minutes after tourniquet release (–12%) ($P<.001$). The HR did not vary significantly during this period in either group.

## PATIENT TREATMENT

Fluid management and the administration of vasoactive drugs are described in Tables 2 and 3 and in **Figure 6**.



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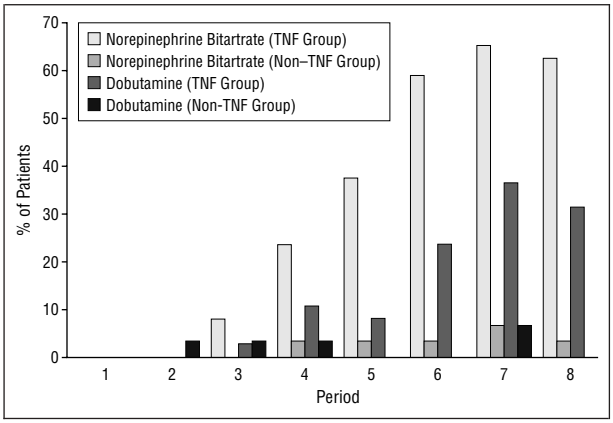


**Figure 6.** Percentage of patients requiring administration of norepinephrine bitartrate and dobutamine in those treated with isolated limb perfusion (ILP) and high-dose chemotherapy, with tumor necrosis factor (TNF) (TNF group), and in those treated with ILP and high-dose chemotherapy, without TNF (non–TNF group). Periods 1 through 8 are defined in the "Hemodynamic Data" subsection of the "Methods" section.

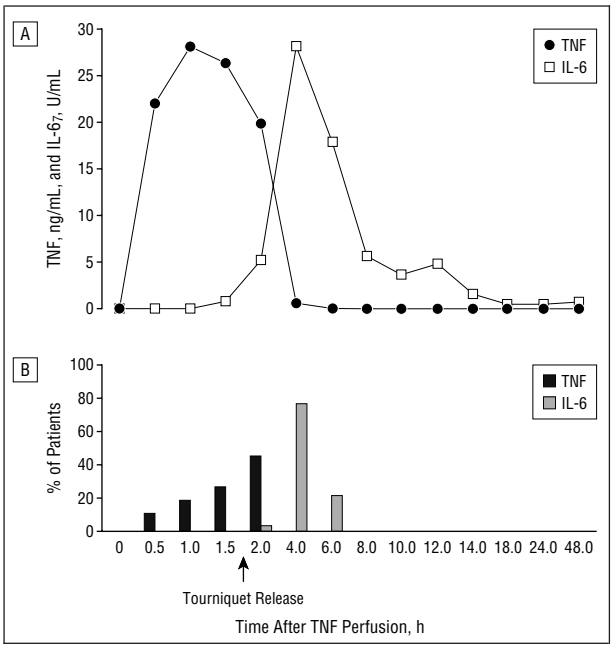


**Figure 7.** A, Median of the systemic levels of tumor necrosis factor (TNF) (measured in nanograms per milliliter) and interleukin 6 (IL-6) (measured in units per milliliter) over time. B, Percentage of patients having their peak level at this point for TNF and for IL-6.

Patients in the TNF group underwent transfusion of more units of red blood cells, had more positive fluid balances, and needed more vasopressive drug support than patients in the non–TNF group, who could always be readily extubated at the end of ILP and only rarely needed intensive care unit (ICU) treatment. All TNF group patients were admitted to the ICU to receive intensive treatment, including mechanical ventilation. All patients survived.

Although dopamine was administered to all but one of the TNF group patients prophylactically, norepinephrine bitartrate and dobutamine hydrochloride were given in both groups only if clinically indicated. (In the TNF group, 7 patients received dopamine alone; 16 received dopamine and norepinephrine bitartrate; 1 received dopamine and dobutamine; 10 received dopamine, dobutamine, and norepinephrine bitartrate; and 3 received dopamine, dobutamine, norepinephrine bitartrate, and epinephrine.) Illustrating the occurrence of hemodynamic alterations necessitating treatment, the percentages of patients receiving these 2 catecholamines are shown in both groups in Figure 6. Within the TNF group, patients who attained a peak systemic TNF level of greater than 100 ng/mL (n=13) were treated on average with significantly more additional catecholamines, besides dopamine, than patients with a peak systemic TNF level of less than 100 ng/mL (n=25) ($P$=.04). In addition, the maximal and the total norepinephrine bitartrate doses administered in the greater than 100-ng/mL TNF group were also significantly higher than in the less than 100-ng/mL TNF group ($P$<.001 [2-tailed $t$ test] and $P$=.001 [Mann-Whitney test], respectively).

## TNF AND IL-6 PHARMACOKINETIC VARIABLES

Systemic levels of TNF and IL-6 were measured in all TNF group patients at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 18, 24, and 36 hours after the TNF bolus injection into the ILP circuit. Maximal systemic TNF levels ranged from 0.03 to 1728 ng/mL. Systemic TNF was detectable in 32 (84%) of the patients during ILP and in 37 (97%) of the patients 2 hours after the TNF injection (ie, shortly after tourniquet release). Ten hours after the TNF injection, fewer than 4 (11%) of the patients still had detectable systemic levels. **Figure 7** illustrates the median systemic TNF and IL-6 levels and the percentages of patients attaining their maximal levels at a given period. The IL-6 levels peaked on average 2.85±0.45 hours after the highest TNF level.

## CORRELATIONS WITH SYSTEMIC TNF LEVELS

The TNF peak systemic level ($r$=0.98, $P$<.001) is strongly correlated to the area under the curve of the TNF systemic level in time.[22] Correlations between the $\log_{10}$ of the peak TNF level and different hemodynamic variables, doses of norepinephrine bitartrate, fluid balance, duration of ICU stay, and IL-6 levels are shown in Table 4. Peak systemic concentrations of TNF correlated well with peak systemic concentrations of IL-6 and with the duration of ICU stay. There were decreases of individual MAP and SVRI values at all TNF plasma concentrations, from 0.03 up to 1728 ng/mL. The minimal readings of MAP and SVRI and the maximal readings of CI reached between TNF perfusion and 36 hours after ILP (periods 3-8) were well correlated to the $\log_{10}$ of the peak TNF level (**Figure 8**). Weak correlations were found for the log of total norepinephrine bitartrate used and for the log of the number of liters of positive fluid balance at 36 hours.

## COMMENT

Our study compared the hemodynamic profiles in 2 groups of patients treated with ILP, with or without TNF,



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to define the specific role of TNF in the early hemodynamic response. We observed 2 distinct hemodynamic responses over time: a tourniquet release effect and a TNF-induced distributive shock.

The TNF group and the non–TNF group experienced an MAP decrease of approximately 10%, 5 and 30 minutes after tourniquet release. The CI significantly increased and the SVRI significantly decreased after tourniquet release (period 4) in the TNF group. In the non–TNF group, the CI also tended to increase and the SVRI to decrease, but not significantly. The previously mentioned changes normalized in the non–TNF group within an hour, while in the TNF group, they persisted for at least 36 hours. It follows, therefore, that tourniquet release induces immediate hemodynamic changes that are overridden by the effects of TNF.

In orthopedic surgery, a thigh tourniquet induces arterial hypertension,[23,24] presumably by reduction of the vascular bed, by intravascular volume expansion due to exsanguination of the operated-on limb, and, most important, because of pain and ischemia.[25,26] When the tourniquet is released, the systolic blood pressure decreases by 15% to 20%, back to the baseline readings or lower; this is related to a decrease of the vascular resistances by an increased total vascular cross-sectional area, reactive hyperemia, and circulation of ischemia-induced metabolites. In a study by Girardis et al,[27] the CI increased and the SVRI decreased in young male patients (mean±SD age, 25.7±6.8; range, 18-38 years) for the first 15 minutes following tourniquet release; these effects are blunted with increasing age.[28,29] During ILP, the tourniquet does not induce ischemia, because this is prevented by the extracorporeal circulation. However, hyperthermia and the perfusion of high doses of cytotoxic drugs may produce vasoactive metabolites that may have the same effect as a tourniquet in orthopedic surgery.

Tumor necrosis factor induced hemodynamic changes that were not observed in the non–TNF group. Being retrospective and nonrandomized, we are aware that our study has limitations. The hemodynamic data were collected at different time points so that we had to define in retrospect the periods during which the recordings were averaged. In addition, the administration of catecholamines and fluids was as clinically indicated and did not obey precise predetermined criteria; this may have influenced the hemodynamic variables. The value of the statistical analysis performed within and between the 2 groups may, therefore, be debated. It can reasonably be assumed that the differences between the TNF group and the non–TNF group would probably have been even greater if no catecholamines had been perfused to the patients in the TNF group.

Despite aggressive fluid loading and vasoactive support, TNF group patients presented for at least 36 hours with tachycardia, hypotension, a high cardiac output, and low systemic resistances, thus mimicking septic shock.[30] No significant changes of the pulmonary artery pressure profile were observed.

This finding of a TNF-induced vasoplegia and loss of vascular tone fits with previous experimental data showing that TNF increases endothelial permeability[31] and induces a vasodilation state with a depressed response to norepinephrine bitartrate[32,33] that persists even after the cytokine is eliminated.[34] The mechanism of this TNF-induced vasoplegia is mainly nitric oxide (NO) dependent,[35,36] although other endothelium-independent pathways have also been incriminated.[37] Tumor necrosis factor causes inducible NO synthase expression,[38-40] so that NO can be produced for several days.[41,42]

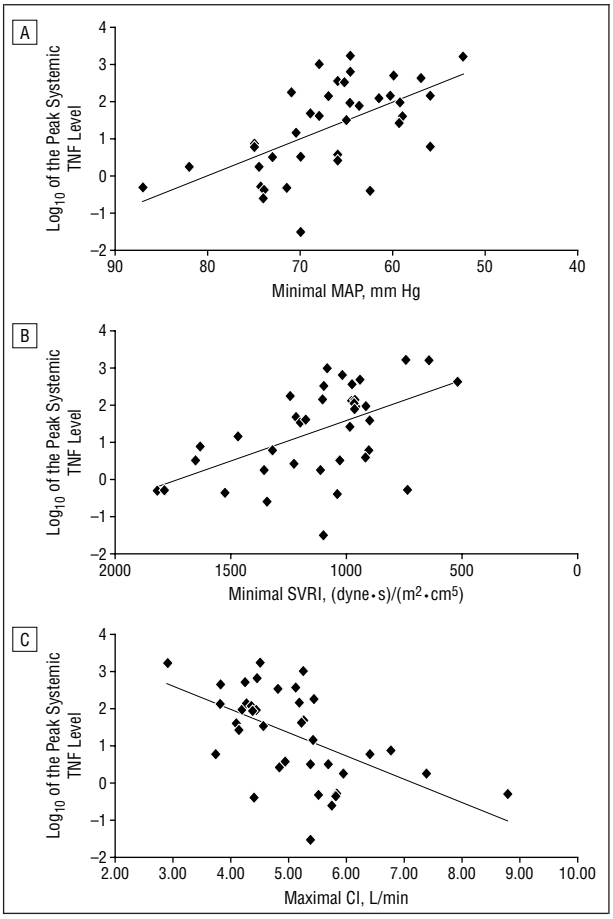


**Figure 8.** Correlation of the $\log_{10}$ of the peak systemic tumor necrosis factor (TNF) level to individual minimal mean arterial pressure (MAP) (A), minimal systemic vascular resistance index (SVRI) (B), and maximal cardiac index (CI) (C) recordings.

Our pharmacokinetic measurements suggest that, in the ILP setting, the presence of TNF in the systemic circulation results from 2 distinct mechanisms: one is leakage from the extracorporeal circuit, and the other is delivery of the cytokine into the systemic compartment when the tourniquet is released. This may happen despite a generous washout of the limb with colloid solutions for more than 10 minutes before the release. In fact, we documented a significant sequestration of radiolabeled albumin in the perfused limb after intensive washout (results not shown), strongly suggesting that TNF was retained in the tissue and released on revascularization. In our TNF group, the peak systemic levels ranged between 0.03 and 1728 ng/mL. The individual peak systemic TNF levels correlated well with the individual minimal recordings of MAP ($r$=0.59) and SVRI ($r$=0.53) and with the individual maximal recordings of CI ($r$=0.55). All but 3 patients in the TNF



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group had an MAP reading below 75 mm Hg and an SVRI reading below 1600 dyne × s × $m^2/cm^5$ at least during one period after TNF perfusion (periods 3-8). The 3 patients who did not experience MAP and SVRI decreases below the norm had low TNF peak levels (0.48-7.36 ng/mL), but most patients with TNF peak levels in this range had an MAP below 75 mm Hg and an SVRI below 1600 dyne × s × $m^2/cm^5$ at least during one period after TNF perfusion (periods 3-8). In addition, patients with high systemic TNF levels (>100 ng/mL) needed significantly more catecholamines and significantly higher norepinephrine bitartrate doses than patients with lower TNF levels, reflecting stronger hemodynamic changes in the former.

Casey et al,[43] in 97 patients with a sepsis syndrome, found detectable systemic TNF levels in 54% of the patients, with a median level of 0.026 ng/mL (range, 0-1.0 ng/mL). In another study[44] on patients with meningococcal disease, all patients who had a systemic level of TNF above 0.1 ng/mL died.

The TNF levels reported in this study are well above these values. This may explain why almost all TNF group patients experienced significant hemodynamic changes. A strong induction of inducible NO synthase by the high doses of TNF during ILP in the vascular bed of the treated limb and subsequent locoregional production of NO may also explain the systemic hemodynamic effects after ILP for as long as 36 hours.

Clinicians should be aware of the potential risks of ILP with TNF because even if leakage is controlled and the limb is rinsed, some TNF will still be released into the systemic circulation after tourniquet release. In addition, even low systemic TNF levels after ILP can induce a septic shock–like syndrome. All patients receiving TNF require, therefore, appropriate hemodynamic monitoring, aggressive fluid perfusion, and, if necessary, the administration of vasoactive amines to minimize the unavoidable, although often mild, vasoplegia. This is not the case for ILP without TNF administration.

In conclusion, our study demonstrates that the release of the tourniquet induces a specific decrease of the blood pressure during ILP with or without TNF administration. This effect is, however, short-lived in the absence of exogenous TNF. It is distinct from the septic shock–like hemodynamic profile observed after ILP with TNF administration, which may occur even at the lowest systemic TNF levels and which requires appropriate hemodynamic monitoring and support.

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### ARCHIVES OF INTERNAL MEDICINE

**Low- and High-Density Lipoprotein Cholesterol and Ischemic Cerebrovascular Disease: The Bezafibrate Infarction Prevention Registry**

*Nira Koren-Morag, PhD; David Tanne, MD; Eran Graff, PhD; Uri Goldbourt, PhD; for the Bezafibrate Infarction Prevention Study Group*

**Background:** Despite increasing evidence that β-hydroxy-β-methyglutaryl coenzyme A reductase inhibitors reduce the incidence of stroke in patients with coronary heart disease (CHD), the associations between blood lipid levels and cerebrovascular disease (CVD) are not clear.
**Objective:** To evaluate whether blood cholesterol level and its fractions are risk factors for stroke in a large group of patients with CHD.
**Methods:** We followed up 11 177 patients with documented CHD who were screened for but not included in the Bezafibrate Infarction Prevention study, a secondary prevention randomized clinical trial of lipid modification, and had no history of stroke for subsequent CVD. During a 6- to 8-year follow-up period, 941 patients were identified as having nonhemorrhagic CVD, of whom 487 had verified ischemic stroke or transient ischemic attack (TIA).
**Results:** Increases in age-adjusted rates of both nonhemorrhagic CVD and verified ischemic stroke or TIA were identified with increasing cholesterol and low-density lipoprotein cholesterol levels, decreasing high-density lipoprotein cholesterol levels, and decreasing percentage of total serum cholesterol contained in the HDL moiety. In logistic regression models, adjusting for clinical covariates, the following odds ratios (95% confidence intervals) were identified for lipid values in the upper vs lower tertile for the end point of nonhemorrhagic CVD: total cholesterol, 1.43 (1.20-1.70); low-density lipoprotein cholesterol, 1.52 (1.27-1.81), high-density lipoprotein cholesterol, 0.84 (0.70-1.00); and percentage of serum cholesterol contained in HDL, 0.69 (0.58-0.83). Similar trends appeared for the end point of verified ischemic stroke or TIA.
**Conclusion:** These findings clearly support the role of total cholesterol and its fractions in prediction of ischemic CVD among patients with established CHD. (*2002;162:993-999*)

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