European Journal of
Nuclear
Medicine

Original article

Continuous intraoperative external monitoring of perfusate leak using iodine-131 human serum albumin during isolated perfusion of the liver and limbs

William C. Barker[1], Mary P. Andrich[1], H. Richard Alexander[2], Douglas L. Fraker[2]

[1] Nuclear Medicine Department, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA
[2] Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

Received 1 June and in revised form 7 June 1995

Abstract. Regional isolated perfusion using tumor necrosis factor (TNF) shows significant promise for treatment of cancer which is limited to limbs or organs. The high toxicity of TNF requires very sensitive real time monitoring of leakage in order to avoid serious patient complications. Human serum albumin labeled with iodine-131 is used with an externally mounted and collimated NaI(Tl) detector to track the leakage of blood from the isolated perfusion blood circuit into the general systemic vascular space. Blood activity levels measured using the monitor demonstrated a very good correlation with blood serum samples taken concurrently with external monitoring. External monitoring can reduce the risks of perfusion leakage intraoperatively with the precision necessary to safely perform isolated perfusion using TNF.

Key words: Perfusion, regional – Tumor necrosis factor – Iodine radioisotopes – Intraoperative monitoring

Eur J Nucl Med (1995) 22:1242–1248

Introduction

Regional isolated perfusion has been performed in many facilities worldwide for the treatment of cancer since 1958 [1]. There have been three stages in the development of regional isolated perfusion: (a) intra-arterial perfusion with an alkylating agent [2]; (b) isolated perfusion using a pump oxygenator [1, 3, 4]; (c) isolated perfusion accompanied by hyperthermia [5]. Regional isolated perfusion allows for the delivery of elevated dose levels of toxic chemotherapeutic agents regionally without the systemic toxicity associated with systemic treatments.

Correspondence to: W.C. Barker

Monitoring of leakage during regional perfusion of the isolated circuit into the systemic circulation has generally been mandatory due to dose escalation of the chemotherapeutics in the perfusate to levels which would be extremely morbid or lethal if given systemically. Many groups have monitored the systemic levels exclusively through the use of blood samples [6–11]. Real time external monitoring used in conjunction with iodine-131 labeled human serum albumin (HSA) was performed as early as 1961 [12]. In those early perfusions using external monitoring, leaks of the perfusate into the systemic circulation ranging from 40% to 80% were not uncommon. Leaks of this magnitude were considered acceptable since the consequences of leakage at dose levels used in those early cases generally were not severe. Another group has suggested the use of two hand-held collimated gamma detectors in leakage monitoring [13]. One difficulty, however, lies in the requirement that the probe positioning be static or consistent throughout the perfusion treatment. The number of photons received by the probe, and thus the signal generated, is geometrically dependent. The resultant leakage measurement could have increased variability introduced by the variability in probe positioning. Furthermore, dose levels of drugs in the perfusion circuit have been escalated such that the danger from leakage is more pronounced. Real time monitoring of systemic levels of toxin with sensitivity to 1% perfusate leakage has been increasingly necessary due to the greater toxicity of the chemotherapeutic agents.

One application which requires highly sensitive leakage monitoring involves the use of perfusion with tumor necrosis factor (TNF) in the regional treatment of melanoma and sarcoma patients [11, 14, 15]. These cases involve delivery of TNF levels to the tumor that are approximately 10 times the maximally tolerated systemic levels. If there is significant leakage (over 10%), the resultant systemic complications could be fatal. With the promise of increased antitumor response rate demonstrated by the use of TNF through regional isolated per-

fusion [14], there exists a need to relate an updated detailed description of an external real time leak monitoring system using currently available equipment.

Materials and Methods

Patients. Forty-eight patients with melanoma or sarcoma of the extremity underwent 53 isolated limb perfusions either with melphalan alone or with a combination of melphalan, TNF, and interferon-γ. In addition, 13 patients underwent isolated hepatic perfusion with TNF and interferon-γ. All patients were treated at the National Institutes of Health Clinical Center after obtaining appropriate informed consent according to institutional guidelines. Five of the above-mentioned 48 patients underwent perfusion of an arm while 43 had perfusion of a leg. The isolated limb perfusions were performed using standard techniques well described in a recent review [15]. Briefly, cannulas were placed in either the axillary artery and vein (for the upper extremity) or the external iliac vessels (for the lower extremity) and then connected to the extracorporeal circuit. The 13 patients with unresectable metastatic disease confined to the liver who had failed all conventional treatment modalities were treated with isolated hepatic perfusion via the hepatic artery after complete vascular isolation of the liver. Venous blood from the intestines, kidneys, and lower extremities was diverted around the liver by a venovenous bypass shunt leading back to the heart via an axillary vein, analogous to that used in liver transplants [16]. This shunt was aided by a centrifugal pump containing an online reservoir with a volume of 40 ml.

Equipment. The monitoring system which has been constructed (Fig. 1) tracks a fixed fraction of the 364-keV gamma rays emerging from ^{131}I-HSA in the cardiac blood pool. A commercially available thyroid uptake system (Model 7350, Canberra Industries, Inc., Meriden, Conn., USA) was used as the radiation detection component of the monitoring system. The uptake system includes a collimated 2″×2″ NaI(Tl) scintillation detector with matching photomultiplier base and preamplifier. The thyroid uptake system is conveniently optimized in terms of detectability and shielding for use with 364-keV ^{131}I gamma rays. High voltage is supplied to the photomultiplier tube by a remotely located 2-kV Bias Voltage Supply (Model 478, EG&G Ortec, Oak Ridge, Tenn., USA). The detector signals generated by the photomultiplier tube are directed to an amplifier (Model 575A, EG&G Ortec) and then to a single-channel analyzer (Model 550A, EG&G Ortec). For the present application, the single-channel analyzer is used as a discriminator to select only signals which correspond to gamma-ray energies greater than 300 keV, thereby effectively isolating the 364-keV photopeak photons. The logic signals generated by the single-channel analyzer are then input to a ratemeter (Model 449-2, EG&G Ortec) which drives a strip chart recorder (Gilson Model BD 40, Kipp & Zonen, Bohemia, N.Y., USA). The ratemeter has a variety of user-selectable rate averaging time constants which allow for smoothing of an otherwise noisy count rate signal.

The NaI(Tl) detector is housed in a flat field collimator (Fig. 2) mounted on an articulated mobile stand. This stand permits easy positioning of the detector in a precordial position over the operating room patient table. Sterile conditions are maintained by enclosing the arm of the detector mount in a sterile plastic bag. The arm is long enough that the remainder of the mobile mount, namely the stand with its associated electronics platform, remains in an adjacent nonsterile area. The collimator is directed such that

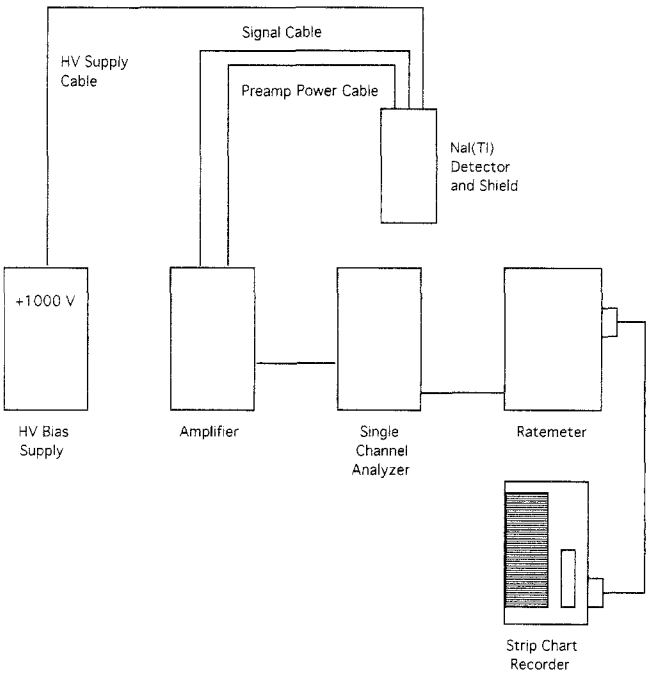

Fig. 1. Block diagram illustrating setup of external monitor

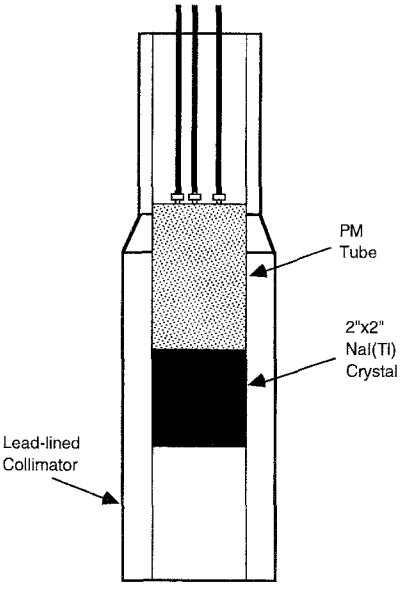

Fig. 2. Diagram showing collimator and NaI(Tl) detector

the detector field-of-view includes the heart only, thus excluding all portions of the perfusate circuit. This orientation allows for maximal shielding of the gamma rays which originate from the perfusate circuit while permitting a maximal amount of systemically originating gamma rays to be detected.

Theory. Isolated limb perfusion utilizing TNF at a dose of 3–6 mg for extremities is limited to a 10% total leak from the perfusion circuit into the systemic circuit during the total treatment intervals as specified by the clinical protocol, which are 60 min for melphalan alone and 90 min for melphalan, TNF, and interferon-γ. To monitor the amount of leakage, two ^{131}I-HSA doses are prepared. First, after surgical isolation of the extremity is accomplished, 20 μCi ^{131}I-HSA is injected into the systemic vascular circuit in order to establish a baseline count level, corrected for room background.

Once the systemic activity distributes throughout the vascular circulation and the activity concentration stabilizes to a constant value (usually within 5–10 min), a 200 μCi ^{131}I-HSA dose is injected into the isolated perfusion circuit. Any leakage from the perfusion circuit into the systemic circulation will manifest itself by an increase in the activity level of the systemic circulation. Quantitatively, a doubling of the activity level in the systemic circulation corrected for room background would indicate a 10% loss of activity from the perfusion circuit. Namely, the systemic circulation would have 40 μCi while the perfusion circuit would be left with 180 μCi. The presumption is that the 10% leakage of ^{131}I-HSA would parallel a 10% leakage of TNF into the systemic circulation.

Stehlin [12] has described a more generalized leakage factor (LF):

$$\mathrm{LF} = \frac{(\mathrm{cpm}_{\mathrm{systemic}} - \mathrm{cpm}_{\mathrm{baseline}})}{\mathrm{cpm}_{\mathrm{baseline}}} \times \frac{V_s}{V_t} \times \frac{D_s}{D_p} \times 100\%, \quad (1)$$

where $\mathrm{cpm}_{\mathrm{systemic}}$=systemic count rate observed during treatment, $\mathrm{cpm}_{\mathrm{baseline}}$=systemic count rate at the beginning of treatment, V_s=blood volume of the systemic circulation (body–limb), V_t=total blood volume (perfusion circuit+systemic circuit), D_s=dose injected into systemic circuit, and D_p=dose injected into perfusion circuit.

This equation was intended to take into account the effect of mixing of radiotracer between the two blood circuits. It assumes that mixing of leakage between the perfused region and the rest of the body involves a complete mixing of the systemic and perfusion circuit volumes. Perhaps this assumption was related to the very high leaks which were observed in those early procedures (40%–80% leaks were common).

It has been our observation that including the volumes in the leakage factor equation for current perfusions, which have a comparatively much smaller leak (less than 10%), is not necessary. Alternatively, we have obtained good results using a modification of Stehlin's equation, namely:

$$\mathrm{LF} = \frac{(\mathrm{cpm}_{\mathrm{systemic}} - \mathrm{cpm}_{\mathrm{baseline}})}{\mathrm{cpm}_{\mathrm{baseline}}} \times \frac{D_s}{D_p} \times 100\%. \quad (2)$$

To compare the leak estimates obtained from the two formulas, consider a case where 20 μCi of radiotracer is injected systemically and 200 μCi is injected into the perfusion circuit. Further, assume that V_s is 4.5 l and that V_t is 6.0 l (typical values when using a pump prime volume of 1.0 l and a limb intravascular volume of 0.5 l). If all of the tracer in the perfusion circuit were to be transferred into the systemic circuit while maintaining the same systemic blood volume (which is, of course, physically impossible but demonstrates the worst-case concentration of tracer), the total activity would be 220 μCi systemically. Stehlin's equation would indicate a leak of 75%. But we know that 100% of the tracer is now present in the systemic circuit. Equation 2, on the other hand, gives the correct value of 100% leak. Therefore, Eq. 2 has been used exclusively in the present study.

To measure the changes in systemic activity concentration, a collimated and shielded external NaI(Tl) radiation detector is placed over the heart in the region of the left ventricle to intercept a fraction, geometrically determined by the collimator, of the gamma rays emitted by the cardiac blood pool. This monitoring method assumes that the cardiac blood pool volume remains relatively constant when averaged over sliding 10-s time intervals (a typical count rate averaging time). All other factors remaining constant, the systemic count rate as determined by the external monitor provides a quantitative measure of leakage from the perfusion circuit into the systemic circuit.

Setup and operation. During the surgical isolation of the limb, the monitor is prepared for use. High voltage is applied to the detector, all settings on the signal processing components are verified and the monitor arm is wrapped in a sterile plastic bag. If desired, verification of the 364-keV ^{131}I energy window can be done using the patient doses prior to injection. The ratemeter of the monitoring system is initially set for a 3-s rate averaging time constant in order to observe the rate peak demonstrating the first pass of the bolus injection through the heart chambers. The recording rate of the strip chart recorder is set to 0.2 mm/min.

When isolation of the limb is complete, the monitor is moved into the precordial position over the heart. The baseline room background level is then measured on the strip chart recorder (typically about 3 counts per second). After the baseline is established, the systemic dose of 20 μCi ^{131}I-HSA is injected intravenously through a central line, making sure that all the dose is completely flushed into the systemic vascular circulation. Within seconds of the injection, the strip chart recorder indicates the passage of the bolus through its first pass in the heart chambers. The bolus peak typically reaches about 250 counts per second. After the first pass of the bolus, the count rate usually stabilizes within 5–10 min to approximately 80 counts per second. Afterward, the averaging time constant is changed to 30 s in order to reduce the noise level in the count rate plot on the strip chart recorder, thus facilitating more accurate estimation of the count rate level.

Once stabilization of the count rate level of the systemic circulation has occurred, the 200 μCi ^{131}I-HSA dose is administered to the isolated perfusion circuit via a port in the perfusion circuit. Immediate changes (within 1 min of injection of the 200 μCi dose) reflect either a misalignment of the collimator such that it views a portion of the perfusion circuit, or the fact that shielding is not adequate. Generally, shielding is not a problem, and collimator alignment is relatively easy to insure. Sustained increases in the systemic count rate (after 2 min following the injection of the 200 μCi dose) indicate leakage from the perfusion circuit into the systemic circulation.

The systemic count rate is monitored carefully for 10 min to verify the degree of isolation of the limb's vascular system. Any leak in excess of 1% over the 10 min period indicates a need for intervention to identify the source and prevent further leakage. A maximum sustained leak rate of 1% per 10 min is tolerated in order to maintain the requirement of no more than a total leak of 10% over the duration of the 90-min perfusion. The 10% total leak threshold refers to the time of actual treatment only. Any leakage prior to the beginning of treatment is noted primarily in order to decide whether to attempt improvement of the isolation. If a significant leak (>10%) occurs prior to the beginning of treatment, appropriate adjustment will have to be made to the interpretation of the strip chart readings. The 10% total leak threshold is determined from the relative activity levels between the systemic and perfusate circuits at the beginning of the treatment period (which occurs with injection of the TNF into the perfusion circuit). Adjustment due to pretreatment leaks can be made by knowing the initial amount of change in the strip chart reading per μCi of administered systemic dose, and then accounting for the amount of remaining perfusate activity. If the leaks are accompanied by large changes in volumes of distribution which would result in dilution of the systemic activity, then allowances for dilution of the tracer should be carried out as well.

Once adequate isolation of the limb's vascular system (the perfusion circuit) has been achieved as demonstrated by a stable systemic count rate, delivery of the desired pharmaceuticals is performed and the 90-min treatment period begins. During the treatment interval, monitoring of the systemic activity level is contin-

ued to verify that the isolation of the limb remains intact. Any sustained increases indicate a need for intervention to improve isolation either by adjusting perfusate flow rates to alter perfusion pressure relative to systemic pressure, or by further surgical dissection. If the 10% leak threshold is reached prior to the completion of the treatment period, treatment is terminated by a rapid flushing of the perfusate from the limb and restoration of systemic blood flow to the limb. A complete flush of the perfusion circuit can be completed within 3–7 min. Monitoring of the systemic activity level is continued until the perfusion circuit has been completely flushed in order to check for leakage caused by the flush process.

For validation of the external monitoring method, blood samples were obtained from the systemic and perfusion circuits. Samples were acquired at the start of the treatment period (0 min) as well as at the 15-, 30-, 60-, and 90-min time points of the treatment period. Systemic samples were obtained from a venous line, while perfusate samples were drawn from the perfusion pump reservoir. If desired, systemic monitoring can continue during restoration of the limb to systemic blood flow for the purpose of checking for residual activity in the limb. In the patients treated in this study, no measurable increase in systemic activity was observed due to residual activity in the limb. Blood samples taken after flushing of the perfusion circuit further confirmed this finding.

Isolated hepatic perfusion. Cases of cancer localized to particular organs can also be treated using essentially the same technique as used in limb perfusion. The equipment setup is identical but with a few procedural modifications. For example, in treatment of metastatic disease restricted to the liver, two perfusion circuits are established. The first is set up to perfuse the liver alone, while the second maintains systemic venous return around the interruption in circulation through the retrohepatic vena cava and portal vein. This second circuit contains a centrifugal perfusion pump which has a small in-line reservoir of approximately 40 ml at the site of the pump. Since monitoring the blood in the chambers of the heart would likely be compromised by scatter from the nearby liver perfusion circuit, the external monitor is instead positioned over this reservoir of the perfusion pump for the venovenous bypass.

Another procedural change involved in liver perfusion monitoring relates to the amount of radioisotope delivered. Since a smaller volume of blood is monitored compared to the intracardiac volume, a larger amount of isotope is used to offset the loss in counts observed by the monitor. Instead of the 20- and 200-μCi ^{131}I doses used in limb perfusion, 40- and 400-μCi doses are used for liver perfusion. However, the additional activity only partially offsets the loss in count rate due to the lower blood volume involved in monitoring. Nevertheless, the resultant count rate is sufficient to discern reliably a leak from the isolated circuit to the systemic circuit of approximately 1%–2%, well within the maximal leak of 10%. Sensitivity could be increased by using a different detector housing which would allow the detector to be positioned much closer to the pump reservoir. That was not done in the present study due to equipment limitations.

Results

In the present study, 54 limb and 13 liver perfusions have been monitored using the described technique. A graph with the monitor readings at 5-min intervals from a select group of patients is shown in Fig. 3 with the resultant leak estimates in Fig. 4. Of the limb perfusion patients monitored, all but one had total systemic leak of no more than 8% during the treatment period. This one patient, randomized to receive melphalan alone, had a calculated leak of 14% (curve number 1 in the figures). Because the toxicities of melphalan are much less severe and more manageable if released into the systemic circulation and the 10% leakage rule for TNF was not observed, treatment was allowed to continue.

Among the other 53 perfusions, 11 had net leaks of 4%–8% (similar to curves 2, 3, and 4 in the figures), 17 had net leaks of 1%–3%, and 25 had either no leak at all or negative net leaks (similar to curves 5, 6 and 7 in the figures). Patients with leaks greater than 1% had higher systemic levels of TNF during and after perfusion. Also, it was observed clinically that those patients with leak greater than 1% required vasopressor support for hypotension, while those with leak less than 1% did not [15]. Only one patient without a discernible leak (by monitor

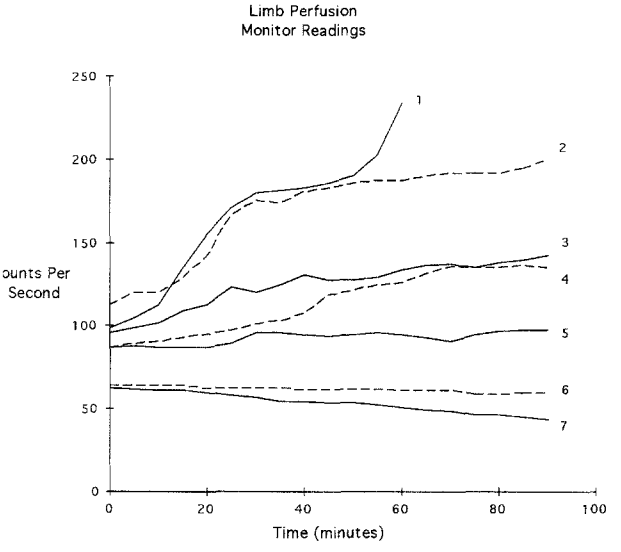

Fig. 3. Typical readings obtained from the external monitor during regional isolated perfusion of a limb

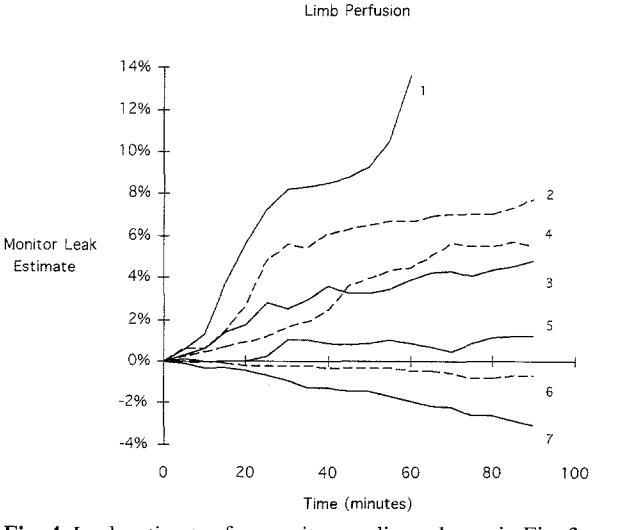

Fig. 4. Leak estimates for monitor readings shown in Fig. 3

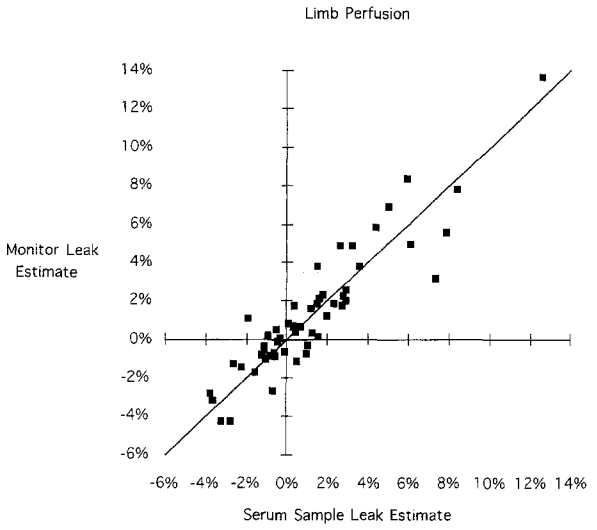

Fig. 5. Correlation between leak estimates from monitor readings and leak estimates from systemic blood serum counts for the 54 limb perfusions. The *solid line* is the line of unity

or by serum TNF levels) required vasopressor support. The etiology of this outcome was not determined. Negative leaks may correspond to reverse leakage from the systemic circulation into the perfusion circuit as manifested by increases in reservoir volumes, dilutional effects from volume replacement into the systemic vasculature during the perfusion, leakage of HSA out of the vascular space, limb or liver uptake of HSA, or in vivo deiodination.

Leak estimates obtained from the monitor readings were correlated with those obtained from systemic blood samples which were taken at the 0-, 15-, 30-, 60-, and 90-min time points, placed on ice and then centrifuged to obtain serum. Leakage was estimated by using the counts obtained by a gamma counter from aliquots of sera. The total net leak estimates as obtained by the monitor are plotted against the serum estimates in Fig. 5. It can be seen from this graph that there is very good correlation between the two methods for leak estimation. It should be pointed out that the level of noise in this graph is predominantly due to variations in our sera sample volumes. In fact, 13 of the 270 time points from 54 limb datasets were excluded due to faulty samples. Other variations are likely in the remaining samples but systematic accounting of these errors was not possible. One likely factor introducing variability into blood sampling is the possibility of incomplete mixing within the blood circuit. Blood samples are drawn from a relatively small volume compared to the total circuit volume. The external monitor, on the other hand, tests a much larger blood volume since it averages over the blood flowing through the heart in a 30-s time period. The monitor readings had a variability of about ±1 out of 25, introducing an error of about ±0.1% in the leak estimates. However, even with the level of variation in the blood sampling, the correlation graph would appear to indicate that the monitor leak estimates are within ±3.0% of the serum samples.

The limb perfusion correlations were also evaluated for patient sex (male versus female), weight, and limb perfused (arm versus leg) to look for any possible distinguishing trends. No significant differences were identified.

In addition to the limb perfusion patients, 13 hepatic perfusion patients treated with TNF were monitored. All cases except one had no leak at all or net negative leaks. The one case with leak had a net leak of 2%. As in the limb perfusions, the patient with leak greater than 1% required vasopressor support for hypotension, while those with leak less than 1% did not. Systemic blood samples were taken at the 0-, 15-, 30-, and 60-min time points in the 60-min treatment period. Again, leakage was estimated using the blood serum counts as well as the monitor data, and correlations were calculated between the leak estimates. The limited number of patients prevented any definite conclusions about the correlations, but the general trend was similar to the limb data shown in Fig. 5. The variability in the monitor data introduced approximately ±0.2% variation in the leak estimates.

Discussion

In the present study, leaks which were observed to be greater than 2% demonstrated excellent correlation to the measured systemic blood sample data. Also, the observed occasional incidence of leaks approaching the 10% maximum threshold for TNF demonstrated the need for continued use of external monitoring in these cases. One group performing regional isolation perfusion, although not with TNF, concluded that leakage monitoring was not necessary since they never observed leaks greater than 1.5% [17]. Our experience demonstrates that at least in the use of TNF it would be unwise to refrain from the use of leakage monitoring. Indeed, one perfusion case in this study (Fig. 6) demonstrated the need to continue monitoring until the limb/organ has been completely flushed. Throughout the treatment period, isolation was excellent and no leak was observed. However, when the perfusionist began to flush the limb, a dramatic increase in systemic activity was observed. The limb flush was immediately terminated until it was found that inadequate venous outflow from the limb led to venous hypertension. Once venous outflow was corrected, the flush was resumed and no further leak was seen.

Variability in the leak estimates for leaks of 2% or less may be the result of many factors. Incomplete mixing of isotope throughout the perfusion circuit, which is mechanically driven, may give slightly variable concentrations over the entire volume of distribution (approximately 1 l). Also, there may be some extraction processes (perhaps related to the hyperthermic warming of the limb, or a breaking down of the isotope labeling) which decrease the amount of circulating HSA over the duration of the treatment period. Dilution of the perfusion

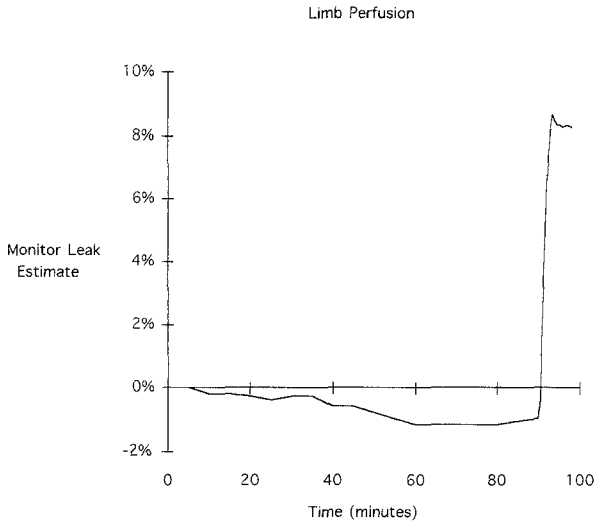

Fig. 6. Leak estimates observed for a case in which major leakage into the systemic circulation occurred during flush

circuit may also occur due to reverse leakage from the systemic circulation into the perfusion circuit. Overall, reverse leakage has not been a major problem since it typically manifests itself by an increase in the volume of the perfusate in a reservoir in the circuit. Reverse leakage from the systemic circulation into the perfusion circuit can be countered by modifying the perfusion pump pressure, and thus the leakage can be minimized.

To make a conservative accounting of effects due to extraction of HSA from the systemic circulation, thus increasing the apparent leakage into the systemic circulation, a linear estimate of extraction could be applied to the monitor data. For example, many of the patients in the current study demonstrated an apparent extraction rate of approximately 1.5% leak equivalent per hour. If it was postulated that all patients by some mechanism extracted at this rate, then the observed leak could be increased at this rate to account for the extraction. Even with this conservative estimate, only one additional patient involved in this study would have approached the 10% maximum threshold. If reverse leakage remains problematic, it could be monitored separately by using ^{131}I-HSA for the perfusion circuit and technetium-99m HSA for the systemic circulation, generating two separable signals for simultaneous monitoring.

An alternative to the use of ^{131}I-HSA with its related waste disposal radiation safety issues may be found in the use of ^{99}Tc-labeled red blood cells (RBCs), or in the use of ^{99m}Tc-HSA. The short half-life of ^{99m}Tc (6 h) and the low energy of the emission photons (140 keV) combine to give radiation safety advantages. On the other hand, the short half-life introduces the requirement to actively correct the monitor data for decay throughout the 60- to 90-min treatment period. Also, the low-energy photons are more subject to attenuation by the patient, thus reducing the counting efficiency and further pressing the need for more activity to obtain adequate signal offset.

Consideration was given in the present study to the use of ^{99m}Tc-HSA but there remained unanswered questions about the relative stability of isotope labeling over the duration of the treatment period. On the other hand, ^{99m}Tc-HSA has been considered useful for external monitoring by other groups [18]. The use of ^{99m}Tc-labeled RBCs continues to be evaluated [10].

In order to perform simultaneous multi-isotope monitoring (e.g., in using a second isotope to track reverse leakage) an alternative equipment setup would require either two distinct amplifier/single-channel analyzer circuits, or a single amplifier coupled with a multichannel analyzer. Also, the data acquired by any of the systems described could be routed to computer rather than or in conjunction with analog strip-chart output by using the appropriate multichannel buffer units or computer-driven multichannel analyzer systems.

In conclusion, it has been demonstrated that continuous monitoring of leakage during isolated regional perfusion using TNF can be adequately performed to allow a 10% maximum leakage to be observed and avoided. Leaks of 1% or less demonstrate variable sensitivity to monitoring while leaks of a more significant level (3% and higher) can be detected very reliably. The method is amenable to modification to allow multi-isotope monitoring as well as the use of alternative tracers, such as ^{99m}Tc-labeled RBCs or ^{99m}Tc-HSA.

References

1. Creech OJ, Krementz ET, Ryan RF, Winblad JN. Chemotherapy of cancer: regional perfusion utilizing an extracorporeal circuit. *Ann Surg* 1958; 148: 616–632.
2. Biermann HR, Byron RL, Miller ER, Shimkin MB. The effects of intra-arterial administration of nitrogen mustard. *Am J Med* 1950; 8: 535.
3. Krementz ET, Creech OJ, Ryan RF, Wickstrom J. Treatment of malignant tumors of the extremities by perfusion with chemotherapeutic agents. *J Bone Joint Surg* 1959; 41: 977–987.
4. Stehlin JS, Clark RL, White EC, et al. Regional chemotherapy for cancer: experiences with 116 perfusions. *Ann Surg* 1960; 151: 605–619.
5. Stehlin JS. Hyperthermic perfusion with chemotherapy for cancers of the extremity. *Surg Gynecol Obstet* 1969; 129: 305–308.
6. Hafstrom L, Hugander A, Jonsson PE, Westling H, Ehrsson H. Blood leakage and melphalan leakage from the perfusion circuit during regional hyperthermic perfusion for malignant melanoma. *Cancer Treat Rep* 1984; 68: 867–872.
7. Lejeune FJ, Lienard D, el Douaihy M, Seyedi JV, Ewalenko P. Results of 206 isolated limb perfusions for malignant melanoma. *Eur J Surg Oncol* 1989; 15: 510–519.
8. Neades GT, Evans WD, Mansel RE. Choice of agent and prediction of systemic toxicity in isolated hyperthermic limb perfusion. *Reg Cancer Treat* 1991; 3: 252–255.
9. Hoekstra HJ, Naujocks T, Koops HS, et al. Continuous leakage monitoring during hyperthermic isolated regional perfusion of the lower limb: techniques and results. *Reg Cancer Treat* 1992; 4: 301–304.

10. Alexander C, Omlor G, Berberich R, Gross G, Feifel G. Rapid measurement of blood leakage during regional chemotherapy. *Eur J Nucl Med* 1993; 20: 187–191.
11. Lejeune FJ, Lienard D, Leyvraz S, Mirimanoff RO. Regional therapy of melanoma. *Eur J Cancer* 1993; 4: 606–612.
12. Stehlin JS, Clark RL, Dewey W. Continuous monitoring of leakage during regional perfusion. *Arch Surg* 1961; 83: 165–171.
13. Sardi A, Minton JP, Mojzisik C, et al. The use of a hand-held gamma detector improves the safety of isolated limb perfusion. *J Surg Oncol* 1989; 41: 172–176.
14. Lienard D, Ewalenko P, Delmotte JJ, Renard N, Lejeune FJ. High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. *J Clin Oncol* 1992; 10: 52–60.
15. Thom AK, Alexander HR, Andrich MP, Barker WC, Rosenberg SA, Fraker DL. Cytokine levels and systemic toxicity in patients undergoing isolated limb perfusion with high-dose tumor necrosis factor interferon gamma and melphalan. *J Clin Oncol* 1995; 13: 264–273.
16. Shaw B Jr., Martin DJ, Marquez JM, et al. Venous bypass in clinical liver transplantation. *Ann Surg* 1984; 200: 524–534.
17. Goldberg ME, Rosenblum HM, Seltzer JL, Rosato FE. Isolated regional perfusion; anaesthetic technique, monitoring and blood replacement. *Can Anaesth Soc J* 1984; 31: 552–558.
18. Kroon BB. Regional isolation perfusion in melanoma of the limbs; accomplishments, unsolved problems, future. *Eur J Surg Oncol* 1988; 14: 101–110.