JECT. 2002;34:130–143
The Journal of The American Society of Extra-Corporeal Technology

Review Article

Isolated Limb Perfusion: A Literature Review

Jennifer Schaadt, MS, MBA, CCP;* Richard Crowley, BS, CCP;† Danielle Miller, MD, PhD;‡ Maureen Kavanah, MD**

**VA Hospital West Roxbury, Massachusetts; †Boston Medical Center, Boston, Massachusetts; and ‡Harvard Medical School, Cambridge, Massachusetts*

Abstract: Through patient education, biological research, and technological advances, the rate of many cancers in the United States of America is declining. However, the incidence of melanoma is rising steadily, as are the efforts and resources allocated to its treatment. Isolated limb perfusion, ILP, is a standard of care for treating recurrent malignant melanoma confined to a limb. Although an extracorporeal procedure, only a small percentage of perfusionists are experienced regarding ILP's indications and performance techniques. Use of ILP may increase as the incidence of melanoma increases. This two-part review is designed to familiarize the perfusionist with the procedure and the disease it treats. Part I reviews the history of isolated limb perfusion, the diagnosis and classification of malignant melanoma, and the applicability of ILP in its treatment. Part II details a procedural overview and technical considerations of the therapy from the perfusionist perspective. The review concludes with patient selection, outcomes, and the future of ILP as well as other applications for the hyperthermic regional delivery of chemotherapy using extracorporeal technology. **Keywords:** malignant melanoma, isolated limb perfusion, extracorporeal circulation hyperthermia, chemotherapy. *JECT. 2002;34:130–143*

PART I

Since 1958, isolated limb perfusion (ILP) has been used in patients with recurrent metastatic malignant melanoma, (MM), confined to an extremity (1). ILP involves the treatment of the cancerous limb with cytotoxic agents in conjunction with high oxygen tensions and hyperthermia. Over the past two decades, little has changed regarding the indications, theory, and execution of this therapy (2). Nevertheless, significant improvements in the technology and opportunities for its application will occur, because the incidence of melanoma has been doubling every 10 years (3).

HISTORY

Krementz and Creech performed the first experimental ILP studies at Tulane University School of Medicine in 1956 (1). Their goal was to increase the chemotherapy dose perfused into an isolated limb without incurring systemic toxicity. By 1957, the researchers succeeded in perfusing six to ten times the routinely used concentration of chemotherapy by using a bubble oxygenator and extracorporeal circulation (ECC) into the isolated limbs of animals. Krementz and Creech used a bubble oxygenator based upon the hypothesis that high partial pressures of oxygen (PaO_2s) would enhance the tumorcidal effect of the chemotherapy, much like the irradiation of cancer cells in a hyperbaric chamber (4). Their research was tested clinically 2 years later in 1958, when they performed the first procedure in New Orleans on a 76-year-old male. This patient refused an amputation for a recurrent MM of his left lower leg but agreed to undergo this experimental therapy. The procedure was a success; the patient remained cancer free, dying 16 years later of old age at 92 (1).

In Europe Cavaliere (1967) (5) and associates demonstrated the selective lysis of cancer cells using hyperthermia obtained by ECC and an oxygenator. However, this group achieved good responses in limb melanomas and soft tissue sarcomas perfused at 41 to 42°C, for 4 h without the addition of chemotherapy.

Five years later, investigators combined hyperthermia and chemotherapy in ILP procedures. It was previously

Address correspondence to: Jennifer Schaadt, MS, MBA, CCP, 4522 Woodman Avenue Pat C 317, Sherman Oaks, CA 91423. E-mail: jschaadt@stm.com
Received July 12, 2001; accepted January 8, 2002.

understood that hyperthermia alone destroyed cancer cells, in addition to potentiating the doses of chemotherapy, and vasodilatating the perfused vasculature (1, 5). A hypothesis was proposed to combine chemotherapy with heat and high PaO_2s to enhance the selective uptake of cytotoxic agents subsequent to increased tumor metabolism and blood flow (1). The clinical outcomes of this hypothesis were successful and proved to be both safe and effective in treating recurrent MM of the limb.

ILP's primary advantage is the ability to deliver high-dose chemotherapy selectively to treat the melanoma and reduce the need for an amputation and systemic exposure to cytotoxic agents. Nevertheless, there are distinct limitations associated with this procedure. The procedure is complex and requires essential extracorporeal equipment, unavailable in many institutions. ILP also demands the expertise and availability of a specialized team experienced with the procedure. In these instances, patients eligible for this therapy may not receive it because of geographic difficulties and inability to gain access to qualified centers.

In the U.S., a unique disadvantage exists concerning the regulatory process of the cytotoxic agent delivered during ILP. Melphalan (L-phenylalanine mustard) is and has been the worldwide drug of choice for ILP (6, 7). Until recently the U.S. Food and Drug Administration (FDA) had not approved Melphalan for regional chemotherapy, although the drug was approved for the intravenous treatment of multiple myeloma (1). In 1992, melphalan was granted orphan drug status for use in ILP by the FDA's Office of Orphan Product Development (8). A drug may receive this status and be approved for either routine or experimental use. In the U.S., insurers limit their reimbursement policies for procedures using orphan drugs (8). As a consequence, although the treatment has been widely used internationally for 39 years, some U.S. insurers considered ILP with melphalan to be experimental and, therefore, designated as nonreimbursable (1).

SKIN CANCER OVERVIEW

Incidences of mast cancers are decreasing; however, skin cancer is the fastest growing cancer in the US (3, 9). Two types of skin cancers exist, malignant melanoma and nonmelanoma skin cancers. Malignant melanoma accounts for 4% of all skin cancers and develops from melanocytes, the pigment-producing cells of the epidermis. The nonmelanoma skin cancers consist of basal cell and squamous cell carcinomas. Basal cell carcinomas are believed to arise from the hair follicle and represent 75% of all skin cancers (10–12). Squamous cell carcinoma originates from genetically altered keratinocytes of the epidermis and makes up the remaining 20% (12, 13). Although the incidence of melanoma is under 5%, it is the most lethal of all skin cancers, accounting for 79% of all skin cancer deaths (9, 14).

MELANOMA RISK FACTORS

International epidemiologic studies suggest exposure to ultraviolet radiation as a major factor in the development of MM in fair skinned individuals (15). Although the exact quantitative and qualitative nature of sun exposure has not been elucidated, it is thought that intermittent and intense exposure to solar radiation with subsequent sunburns markedly increases an individual's risk for developing MM. Additional risk factors include light skin pigmentation, prominent freckling, the inability to tan, blonde or red hair, and blue or green eyes (16). Additional variables associated with sun exposure include occupation, geography, latitude, and socioeconomic status (17). The latter variable explains the historic reference to MM as the "rich man's sarcoma" or "rich man's disease." Years ago, people of higher socioeconomic status probably had more leisure time and were able to spend long vacations in warm, sunny climates with intermittent and intense sun exposure.

Patients with familial melanoma are at high risk for developing MM and comprise approximately 8–12% of MM cases (15). MM in childhood and adolescence is a rare event, accounting for 1–4% of all newly diagnosed cases in patients under the age of 20 (18, 19). MM can arise on any area of the skin, and its incidence is equal in men and women. Melanoma is rare in blacks and most frequently involves the soles, palms, and nail beds (acral sites). In whites, women tend to develop melanomas more often on the extremities; whereas, men frequently develop melanomas on the trunk, head, and neck regions (15). In cases where MM is confined to a limb, roughly 75% of the MM cases occur on the lower extremities (3).

DIAGNOSIS

Diagnosis of MM is done clinically by physical examination and dermatoscopy, as well as by histologic examination. Clinical diagnosis of melanoma relies on visual assessment of several gross morphologic features of a pigmented lesion. Signs of malignancy include asymmetry, large size (>6 mm), multiple shades or colors, and irregular borders (20) (see Plates 1–4). Additional warning signs include itching, ulceration, bleeding, and the development of satellite lesions. Because early detection of an MM yields a better prognosis, the American Academy of Dermatology promotes regular self-examinations of the skin for melanoma using the ABCDs (20, 21). (The ABCD acronym stands for Asymmetry, Border, Color, and Diameter.) If, on examination, one or more of the ABCDs is

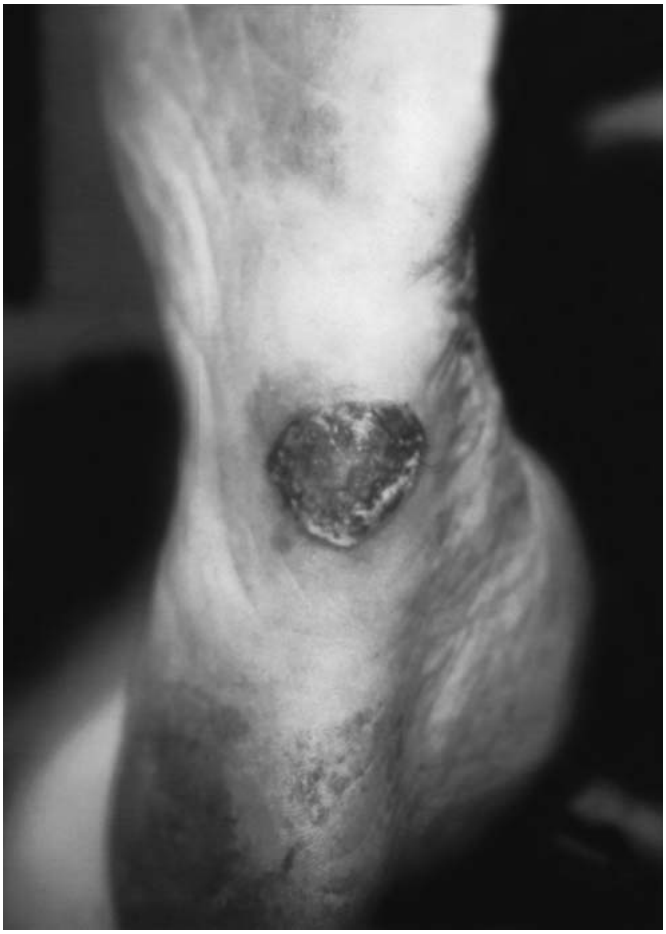

Plate 1. An acral malignant melanoma of the sole of the right foot in an 87-year-old female. (Reproduced with physician permission.)

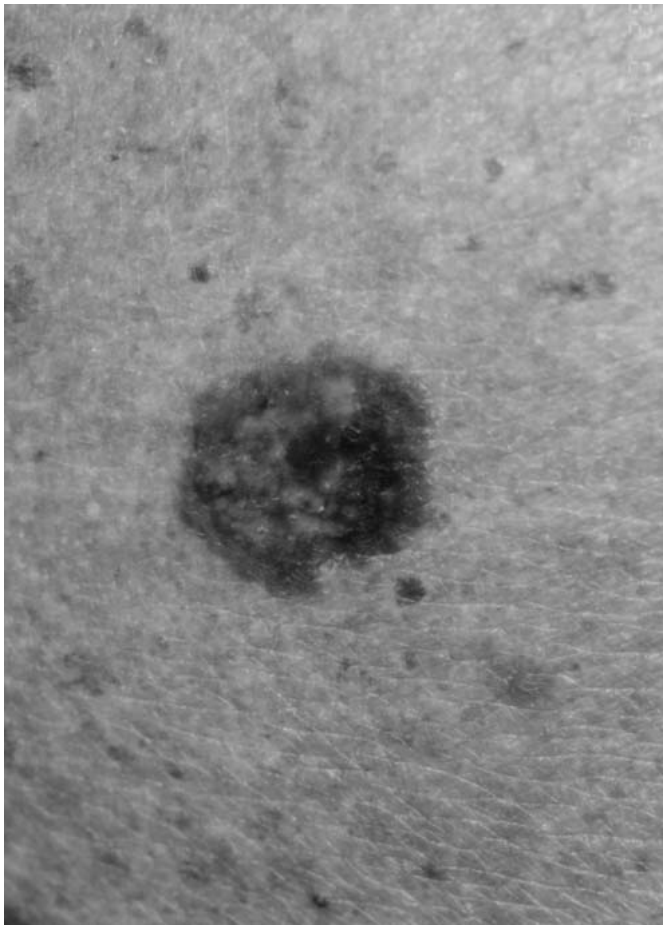

Plate 2. An irregularly pigmented brown–tan–black asymmetric macule, (flat lesion <1.0 cm) representing a malignant melanoma. (Reproduced with physician permission.)

detected, a physician should be seen immediately. Further work-up entails a full thickness skin biopsy of any suspicious lesions (preferably by local excision), which is then sent to pathology for classification and microstaging (15).

CELLULAR CLASSIFICATION OF MELANOMA

Four primary classifications of MM exist and are defined by their gross characteristics and microscopic features, as well as growth patterns: lentigo maligna melanoma (LMM), superficial spreading malignant melanoma (SSMM), nodular malignant melanoma (NMM), and acral lentiginous malignant melanoma (ALMM). A fifth miscellaneous class is occasionally referred to in some texts as spindle cell, desmoplastic, and other unusual types of MM (15). Differences between the primary cellular melanoma classifications include the age at detection, gender, race, frequency of occurrence, lesion site, and histopathology. Specific characteristics associated with each melanoma classification are detailed in Table 1. Important histological attributes influencing the microstaging of MM include the presence or absence of ulceration, regression, and intraepidermal component, vascular invasion, lymphatic invasion, microscopic satellites, as well as the mitotic rate, the cell type, and the precursor lesion.

CLINICAL STAGING

Accurate clinical staging is essential for the management and development of the appropriate treatment plan for patients with MM. The tumor, node, metastases, (TNM) classification is used to provide patients with a prognosis regarding their life expectancy (9). The American Joint Commission on Cancer, (AJCC), has a four-stage classification system involving the evaluation of the local tumor site, (the thickness of the primary MM), evaluation of the adjacent skin, (the presence or absence of in transit metastases), involvement of regional lymph nodes, and the presence or absence of metastases involving distant organ systems (17). Stage I refers to local disease with a primary lesion. Stage II comprises the primary tumor

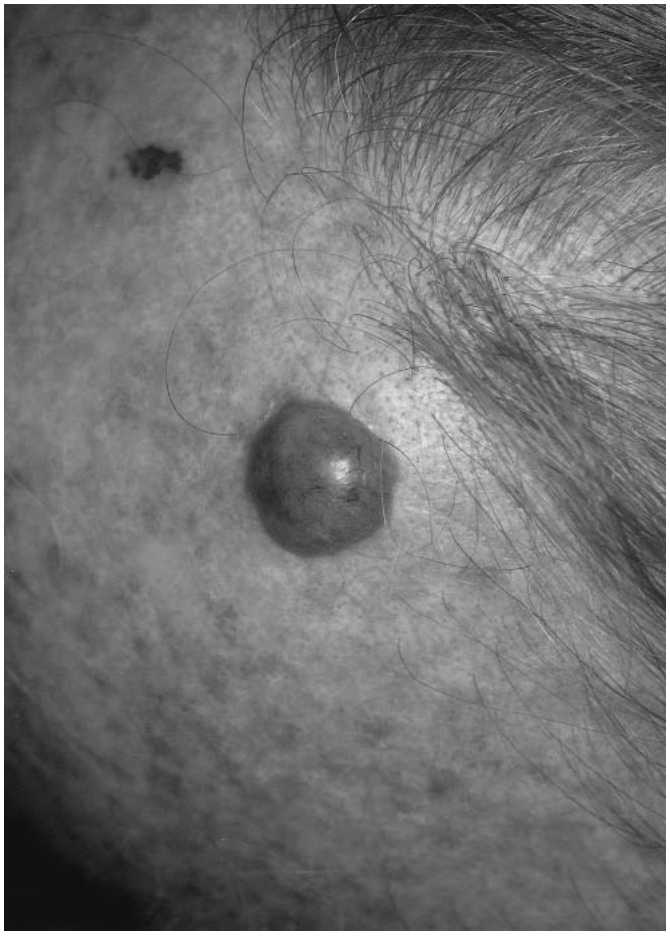

Plate 3. This patient was referred for an excision of a new cyst on the scalp of 1-month duration. Biopsy revealed metastatic malignant melanoma. He had been diagnosed with a malignant melanoma of the leg 9 years earlier and had been subsequently lost to follow-up. (Reproduced with physician permission.)

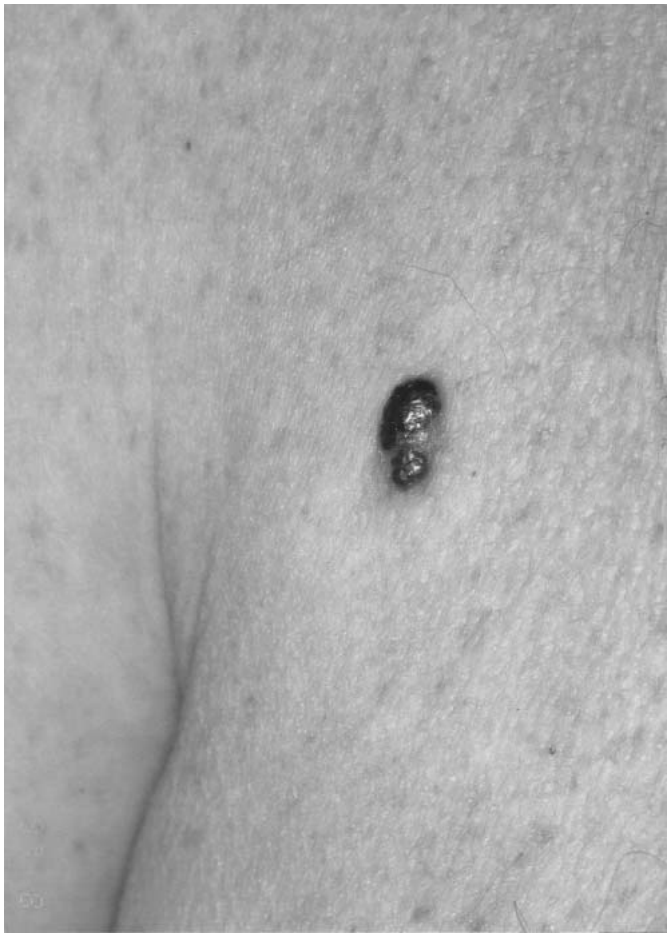

Plate 4. An irregularly pigmented brown–black papule, (raised lesion <1.0 cm), which revealed a melanoma on biopsy. (Reproduced with physician permission.)

site, in addition to the presence of satellite tumors 3 cm from the primary lesion. In Stage III, the MM has metastasized to the regional lymph nodes draining the tumor area, and Stage IV denotes distant metastasis.

Similar to the AJCC system is the M.D. Anderson staging system. This system is more detailed and is, therefore, the primary classification system used for staging melanomas and establishing treatment modalities (22).

Stage

I. A. Primary only
   B. Primary excised
   C. Multiple primaries
II. Local recurrence within 3 cm of primary
III. A. In transit recurrence; within blood vessels or lymph channels, no nodes
   B. Regional nodal recurrence (no in-transit)
   C. In-transit and regional nodal recurrences
IV. A. Distant cutaneous metastases
   B. Distant visceral metastases

MICROSTAGING: CLARK AND BRESLOW

The microstaging of MM is determined in part by the anatomic level of local invasion and is described as a Clark's Level I–V (23).

Level I epidermis involvement only (also called in situ melanoma)

—Level II invasion of the papillary dermis
—Level III invasion fills the papillary dermis
—Level IV invasion into the reticular dermis invasion
—Level V invasion into the subcutaneous fat

The Breslow classification refers to the depth of local invasion by the primary tumors and is measured in mm (24). The depth of the melanoma is measured from the top of the granular layer of the tumor to the point of deepest invasion (see Figure 1).

—Thickness .75 mm or less
—Thickness .76 mm to 1.5 mm
—Thickness 1.51 mm to 4.0 mm (considered high risk)
—Thickness 4.0 mm or greater

Table 1. Characteristics of the four cellular melanoma classifications.*

	Lentigo	Superficial Spreading	Nodular	Acral
Frequency	5%	70%	15%	10%
Site	Face	Men torso Women extremities	Any site	Soles, palms, nail beds
Mean diagnosis age	70s	mid-40s	Late 40s	60s
Population/pt characteristics		Most common in light-skinned population		Most common in Asians and blacks; rare in whites
Visual features	Usually flat brown/black freckle-like	Smaller raised lesion pink, blue, brown, white, gray	Brown/black 5% are flesh colored	Flat lesions, brown, black
Growth pattern	Radial growth irregular shapes	Glossy surface notched/scalloped borders	Rapid evolution vertical growth nodular-like	Aggressive growth, irregular borders
Duration before diagnosis (yrs)	5–50	1–7	Months	1–10

*Data presented in this table from references (1, 2, 15).

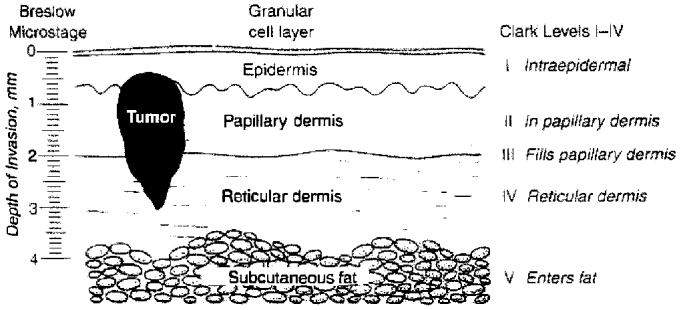

Figure 1. Breslow classification. Reprinted with permission from Clark WH, et al. The histogenesis and biologic behaviors of primary human malignant melanomas of the skin. 1969; Cancer Res. 29:705.

MANAGEMENT

Management of MM depends on the TNM staging at the time of diagnosis, which, in turn, is determined by the Breslow Microstage and the Clark's level of local invasion, in addition to the location of the primary lesion, patient age, the recurrence rate, and previous therapy modalities used. The appropriate treatment strategy for a MM can be one or more of the following: chemotherapy, biologic therapy utilizing immune modulators, radiation therapy, surgical excision, amputation, sentinel node mapping and selective lymph node dissection, and ILP (15). Because of the specificity of this article, only ILP and ILP-related options are discussed.

ILP OVERVIEW

The standard limp perfusion protocol entails the extracorporeal delivery of a high-dose chemotherapeutic agent intra-arterially to the affected limb using 40–42°C oxygenated blood (1–7, 26, 27). After the initiation of isolated limb bypass, leaks between the limb and systemic circulations are ascertained using a variety of techniques (1, 7). With adequate vascular isolation, chemotherapy is given intra-arterially and circulated at an elevated temperature for 60 min. When the perfusion has been completed, the perfusate is diverted to a waste bag, and the limb is washed out with 3–5 liters of crystalloid solution. Once the effluent is almost clear, the limb is infused with 0.5–2.5 liters of a low molecular weight dextran. Perfusion is then discontinued, vascular repair is performed, and any other planned surgical procedure on the melanoma and or regional lymph nodes is done.

MELANOMA AND ILP

Presently, three indications exist for isolated limb perfusion for the treatment of MM and sarcoma: (1) therapeutic ILP for locally and regionally advanced malignant melanoma, including local recurrence of MM, satellitosis, and intransit metastases; (2) ILP is also indicated in locally advanced soft tissue sarcoma not amendable to surgical resection; (3) ILP can be used as an adjunct to local excision of a high-risk primary, or recurrent MM (2). The first indication is the most widely used and accepted indication for ILP (25).

Surgical excision is typically the first choice of treatment of MM (15) and may be done in combination with ILP. However, surgery may not be feasible if there is a large area of involvement with multiple lesions and satellites. Systemic therapy is widely employed as well, although its use exposes other organ systems to cytotoxic agents and does not usually benefit local/regional disease. Relative to amputation, ILP offers a better quality of life over amputation.

POST-ILP OUTCOMES

Early detection of MM increases melanoma cure rates. Long-term survival figures of 92% for a localized stage I melanoma decline sharply to less than 5% for metastatic stage IV disease (15). Prognostic indicators for MM include gender, age, tumor site, tumor thickness, level of invasion, and cellular classification. The long-term prognosis is slightly worse in males. Additional factors negatively affecting outcomes include advanced age, acral sites, increasing tumor thickness, and deeper dermal levels of invasion.

Complete remission rate for patients receiving ILP for melanoma with multiple recurrences or in transit metastases ranges from 40–90% (6). The variance in survival outcomes are attributable to the prognostic indicators mentioned above as well as the presence or absence of undetected micrometastatic systemic disease and its management (26). Postlimb perfusion cumulative survival rates according to their prognostic indicators and histology are found in Tables 2 and 3.

PART II

Isolated limb perfusion is an extracorporeal procedure with many facets identical or similar to those in cardiac surgery. However, unlike cardiopulmonary bypass, ILP has two circulatory systems, limb and body. The circulations must remain isolated, because the extent of leakage between systems determines the severity of systemic toxicity. Leaks are directional and may occur in either direction; blood either shifts from limb to body, often termed a "systemic leak," or from body to limb. Systemic toxicity results if significant leakage of the limb's chemotherapy-enriched perfusate leaks goes into the systemic circulation. Similarly, a compromise of the patient's hemodynamic stability results if considerable systemic blood volume goes into the limb.

Table 2. Survival rates and prognostic indicators in 1088 ILP Krementz 1957–1992.*

Stage	No. Patients	Cumulative Survival 5 yr%	10 yr%	15 yr%	20 yr%
I. Primary					
Male					
Upper	75	86	71	59	56
Lower	84	62	57	47	43
Total	159				
Female					
Upper	105	88	78	73	66
Lower	194	89	77	69	62
Total	299				
All with RLND†	310	87	74	66	63
All without RLND	148	76	61	52	42
Totals stage I	458				
II. Local recurrence					
Male	12	83	31	17	17
Female	24	78	78	69	61
Totals stage II	36				
III. Regional metastases					
Intransit	143	36	30	22	18
Nodes	180	45	39	32	30
Intransit and nodes	145	23	17	10	10
Totals stage III	468				
IV. Distant metastases					
Total stage IV	126				
Total all stages I–IV	1088				

*Data presented in this table from reference (1).
†RNLD: radical lymph node dissection.

Table 3. Survival rates in 488 ILP patients by histology (*all stages).

	Lentigo	Superficial Spreading	Nodular	Acral Lentiginous
Number of patients	23	192	129	90
Post ILP survival rates @ 5 years	91%	79%	63%	48%
Post ILP survival rates @ 10 years	77%	68%	54%	34%

*Data presented in this table from reference (1).

Careful and constant communication between team members must occur throughout the procedure. This review will familiarize the clinician with these system dynamics and the technical aspects of procedure. Review topics include anesthetic overview, perfusion equipment and circuit prime, anticoagulation requirements, cannulation, pump run logistics, temperature management, chemotherapeutics, leak assessment, and washout.

ANESTHETIC OVERVIEW

Patients arrive in OR for limb and systemic arterial and central venous line insertions. (Swan Ganz catheters are not warranted in these cases). General anesthesia is induced followed by endotracheal intubation (28). After intubation and the institution of mechanical ventilation, baseline blood gases and an activated clotting time test (ACT) (or heparin concentration) are drawn. Ideally, all ventilated gases should be heated and humidified to prevent potential heat loss via the respiratory tract (7). Anesthesia is maintained with general anesthetics (7, 28). Conventional patient monitoring includes ECG, pulse oximetry, CVP and arterial pressures, urine output, and tidal CO_2, nasopharyngeal and bladder temps (7, 28).

PERFUSION EQUIPMENT

Equipment consists of a roller pump, or centrifugal pump, oxygenator with a gas source, heater cooler capable of reaching 42°C, open or closed venous reservoir, and/or a cardiotomy. The literature documents the use of either a centrifugal pump or a roller pump as the arterial pump. Pump selection is up to the perfusionist and equipment availability; however, valid arguments exist in favor of both types. Centrifugal pump are afterload sensitive; thus, in the event of increased resistance, flow decreases. Because the primary goal is to deliver high blood flow to the tumor in a hyperthermic environment, an occlusive roller may be better suited for this application. Nevertheless, use of centrifugal pumps affords an element of safety in the event of inadvertent circuit pressurization.

The tubing circuit line lengths should be minimized to lower prime, yet assure safety and sterility. Quarter-inch tubing and a pediatric oxygenator is an option for upper

arm perfusions, provided the oxygenator can sustain PaO_2's greater than 400 mmHg at the necessary flow rates (4). The same requirement applies for leg perfusions, either an adult or pediatric oxygenator can be used, with a 3/8-in tubing circuit. Additional circuit components include a sample manifold for intra-arterial chemoadministration, a waste bag, Y connected into a 3/8-in venous line for washout, and perfusate temperature probes (see Figure 2). Safety devices include a flashlight, hand cranks, cable ties, and back-up supplies, etc. The literature does not elucidate the necessity of arterial filters, level alarms, air detectors, venous saturations, and in-line blood gas analyzers, hence the use of these devices is at the perfusionists' discretion.

CIRCUIT AND PRIME

The choice of prime varies according to institution. The majority of hospitals use a balanced electrolyte solution, (7, 29–31) with the addition of a unit of packed red blood cells, PRBCs, sodium bicarbonate, and 3000–5000 units of heparin. Many centers advocate using two units of PRBCs for lower limb perfusions, and one European center reports using a 750-cc heparinized whole blood prime (32). Prime volumes range between 700–1200 cc because of the circuit components and line/tubing dimensions.

The use of banked blood is essential in ILP to assure adequate oxygen delivery because of hyperthermia-induced increased tissue metabolism. As in cardiac surgery, the impact of large pump primes and limb hemodilution can be diminished by the use of smaller circuits and retrograde autologous priming (RAP). RAP has been previously described in perfusion literature (33, 34) and involves the retrograde displacement of the crystalloid volume in the pump lines with the patient's blood. Depending on the circuit and recirculation methods, RAP techniques can be successfully applied in ILP cases as well.

ANTICOAGULATION

Two separate circulatory systems, both requiring anticoagulation, exist during ILP. Full systemic herapinization

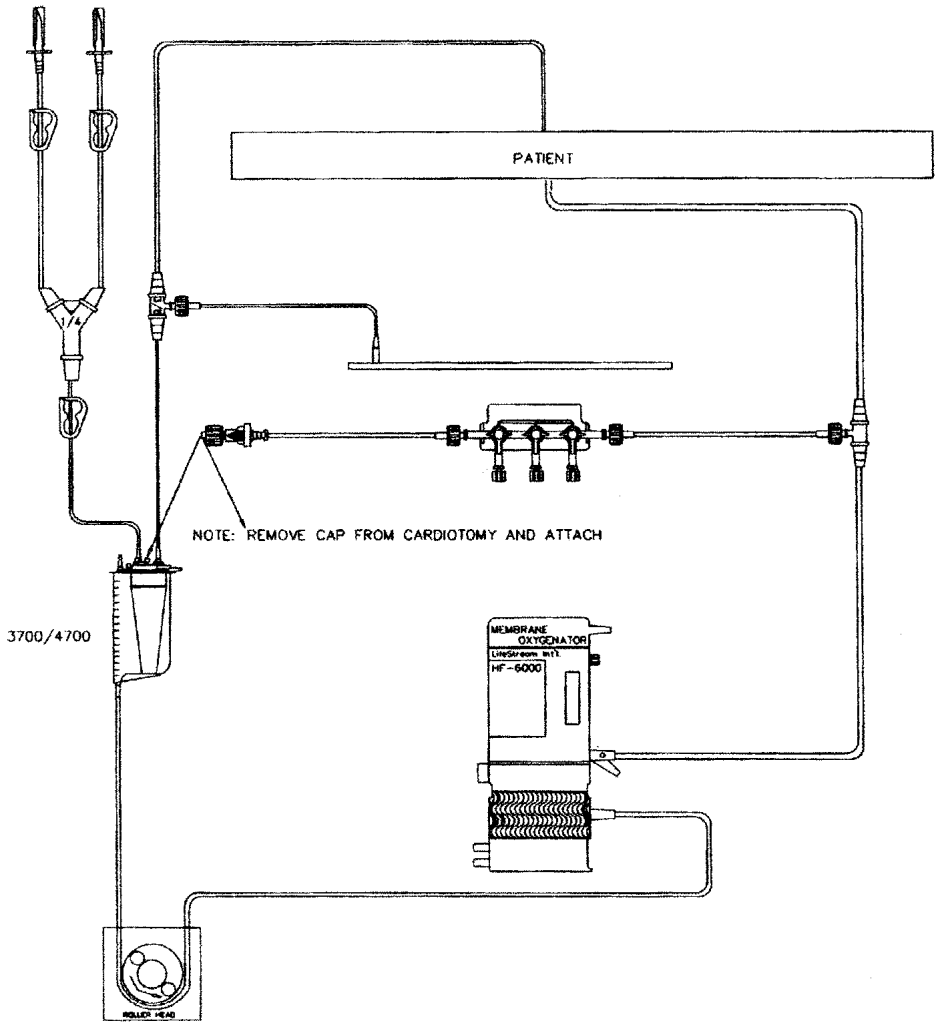

Figure 2. Perfusion equipment.

of the patient must occur before the insertion of vascular cannulas (3, 7, 29, 30, 32). The patient remains heparinized through the entire procedure, because inadequate anticoagulation would potentiate limb circuit clotting in the event of a systemic-to-limb blood leak. Anticoagulation of the limb system is necessary to prevent clotting and limb thrombosis subsequent to contact with foreign surfaces of the extra corporeal circulation (ECC). ACTs are drawn from the limb, and the patient is maintained according to hospital protocols.

Heparin-bonded circuits and cannulae have been successfully used in cardiac surgery for the past decade (35). Some cardiac centers using heparin-coated tubing and cannulas have lowered the heparin dose, targeting ACTs of 250 seconds (34, 36). Correspondingly, perfusionists have successfully adapted this anticoagulation methodology to limb perfusions. Using this technique at the termination of perfusion, the anticoagulation status is not reversed via protamine and allowed to return to normal gradually. This is significant, because the recurrence of the disease in some melanoma patients may subject them to multiple limb perfusions and several protamine exposures. Moreover, one of the complications associated with the ILP is limb thrombosis. In light of this, using low-dose heparin without protamine reversal anecdotally may lessen the probability of thrombosis early in the postoperative period. Under certain circumstances, protamine may be administered in cases where severe bleeding occurs after vascular repair.

CANNULATION/ISOLATION

Cannulation is achieved by means of open and direct arteriotomy and venotomy (3). Patients with melanoma of the thigh or groin are perfused through the external iliac vessels. Lesions of the distal thigh and below are perfused through the femoral vessels. Arm perfusions are performed through the first portion of the axillary artery and vein. Cannula size depends upon the patient's vasculature and the extremity perfused. Lower extremities use venous cannulas ranging from 14–20 Fr, and arterial cannulas from 12–16 Fr. Upper extremities use arterial and venous cannulas ranging from 10–16 Fr (1, 29, 30).

Isolation of the limb from the systemic circulation occurs after cannulation and is one of the key elements in ensuring a successful outcome. Total isolation between systems is vitually impossible because of the presence of deep venous collateral vessels and variations in patient anatomy (7, 28). Consequently, leaks should be minimized and occur in the direction of body to limb. Tourniquets have been used for decades as the sole method of isolation. Tourniquets consisting of one or two loops of Esmarch bandage are placed around the root of the extremity, proximal to the insertion of the perfusion catheters. For lower extremities, the Esmarch bandage is wrapped around a large Steinmann pin placed through the skin and subcutaneous tissue into the iliac crest (3).

However, in an effort to minimize systemic toxicity subsequent to systemic leaks, a change from tourniquet occlusion to a more sophisticated flow balance technique has been developed (28). This technique involves the use of larger lumen cannulas and maintenance of precise arterial and venous gradients between both systems. Vessel permitting, upper and lower limb perfusions use arterial and venous cannula sizes of 20 and 22 Fr. Ideally, the larger the cannula, the better, because success of the perfusion is dependent upon the tumor receiving adequate flows.

In the flow balance technique, isolation occurs by the regulation of pressure gradients between the limb system and the patients system (7, 28). Thus it is imperative that limb arterial and venous pressures are monitored in addition to patient CVP and arterial blood pressure. Limb arterial pressures are measured somewhere distal to the cannulation site, typically the dorsalis pedis for a leg and the radial artery for an arm. Limb venous pressures may be transduced using a venous cannula with a luer port connected to high-pressure monitoring line. The perfusionist regulates limb pressures using flow and tubing clamp placement on the venous line. Systemic arterial and venous pressures are manipulated by the use of vasoconstrictors and the administration of IV fluids by anesthesia (28).

The regulation of flows, pressures, and leakages follow the laws of fluid dynamics where fluid flows in the path of least resistance. In ILP, the paths are the vasculatures of either the limb or body; hence, blood flows from the system of higher resistance or pressure to the lower-pressure system. Theoretically, systemic hypertension in excess of limb arterial pressure causes a shift of systemic blood volume into the limb circuit. Conversely, if the patient's systemic blood pressure decreases below the arterial pressure of the isolated limb, a leak into the systemic circulation can occur. The same principle applies to the venous system as well.

To minimize the amount and ensure direction of the leak, a gradient of approximately 15–20 mmHg must be maintained between the systemic *diastolic* pressure, (*not mean*) and the limb mean arterial pressure (7, 28). Similarly, a gradient of at least approximately 2–6 mmHg must be maintained between the central venous pressure of the body and the limb venous pressure (28). It is imperative, that patient system and limb pressures are continuously observed to make certain detrimental fluid shifts in either direction do not occur. By manipulating limb venous, arterial, and central venous and systemic arterial pressures, adequate flow can be accomplished while maintaining the appropriate systemic and limb arterial and venous gradients (28).

PERFUSION PARAMETERS

The limb flow rate depends on the limb perfused, cannula size and placement, venous return, and limb pressures. No specific guidelines for flow rate calculations have been cited in the literature. Flow rates quoted ranged from 80–400 mL/min for upper extremities and 150 mL/min–1.5 L/min for lower extremities (3, 29, 26, 30, 32).

Although no prescribed flow criteria exist, some centers advocate flow rate calculations based upon the Rules of 9. This rule bases flow rates upon the percentage of cardiac output going to the extremity, with the lower limb receiving approximately 18% of the cardiac output and an upper limb 9%. The majority of ILP patients do not have Swan–Ganz catheters, so cardiac output must be calculated by the Fick equation or estimated based on weight; that is, 50–75 mL/kg or body surface area, 1.8–2.4 L/m squared. Obviously, the latter is a much easier option, albeit less precise. The volume range, 50–75 mL, per kg weight is determined by the clinician's assessment of the individual patient.

For example, a 36-year-old, 60 kg, female has an estimated cardiac output of 3600 mL:

- 60 kg × 60 mL/kg = 3600 mL

Using the rules of 9, 18% of the cardiac output is the required flow for a leg:

- 3600 mL × 0.18 = 648 mL/min

For an arm, 9% of the patients cardiac output is required:

- 3600 mL × 0.09 = 324 mL/min

Following are some key principles to remember regarding the calculation and maintenance of flow rates during ILP.

- Melanoma patients are considerably younger than the typical cardiac patient and will have normal blood volumes.
- They are NOT cardiac patients and have normal cardiac outputs and ejection fractions.
- Tumors have an increased vasculature relative to the native tissue, thus preferential blood flow is to the tumor.
- Local metabolism is increased because of hyperthermia.
- The cannula size selected should meet calculated flow rates.

Limb perfusion is initiated after verifying sufficient anticoagulation. It is best to exsanguinate the limb slowly by the manipulation of the venous clamp while commencing arterial flow. As with any perfusion, the adequacy of venous return must be assessed as well as the arterial line pressure. If venous return is inadequate or there is a sudden increase in arterial line pressure, perfusion must be discontinued, and cannula placement must be examined and adjusted.

Once perfusion is safely established, hyperthermia is instituted. The goal in limb perfusion is to achieve and sustain the target temperatures, maintain consistent venous reservoir volumes, and keep limb pressures less than systemic. Achieving this dynamic equilibrium takes time because of the physiologic effects of warming on both systems.

Hyperthermia will increase the capacitance of the limb's venous system and potentiate collateral vessel expansion. Thus, even with a constant arterial flow, venous reservoir levels decrease over time. Adequate reservoir levels may be sustained by the periodic addition of crystalloid into the pump circuit. Moreover, changes occur within the patient, because the normal physiologic response to increasing temperatures is tachycardia and increased cardiac output.

Blood gas management involves maintaining PaO_2s over 400 mm Hg for tumorcidal enhancement and normal $PaCO_2$s. The hematocrit will be dependent upon the patients starting hematocrit and the constituents of the pump prime. Electrolytes remain normal with the exception of a low ionized CA^{++} attributable to the high proportion of banked blood in the limb circulation. Limb and patient ACTs are drawn accordingly. The pH of the limb system should be normal, *before* the administration of chemotherapy and will subsequently rise to abnormal levels after chemo delivery.

Further responsibilities for anesthesia personnel during the perfusion involve the continuous observation of four to six limb temperatures (1). Additional temperature monitoring ensures the adequacy of hyperthermia and is standard practice in all ILP procedures. However, methods used to ensure limb isolation and leak assessment vary. Depending on the institution, the anesthesiologist may be requested to insert arterial and venous lines in the affected limb, as referred to in the cannulation and isolation section, and/or the measure the concentration of expired anesthetic gases to be discussed in the following leak assessment section (28).

LEAK ASSESSMENT

Serious intra- and postoperative toxicities result from the systemic leakage of the perfusate. Thus, before any administration of chemo, the degree and direction of shunting should be determined. Preferably, the technique for leak assessment should be easy to perform, quantifiable, occur in real time, be repeatable and economically feasible. Unfortunately, one single technique does not possess all these attributes. Current techniques include fluorescein dye, radiolabeled tracers, dye dilution, plasma

melphalan levels, and exhaled desflurane levels. Other secondary leak indicators include temperature changes, reservoir level changes, ACTs, and hematocrit changes.

The traditional circuit to system leak method involves the injection of fluorescent dye into the pump before the introduction of the cytotoxic agent. Three hundred mg of fluorescein is added intra-arterially via the pump's manifold and circulated for several minutes. Because of the increase vasculature of the tumor, it will absorb a proportionately larger amount of dye. OR lights are turned off, with the exception of the reservoir light, or flashlight on the pump and anesthesia monitors. An ultraviolet (UV) light source, Woods lamp, is used to illuminate the dye absorbed by the vessels and tissues. With good isolation and perfusion, illumination of the tumor's dense vascular tree is present along with a line of demarcation at the level of cannulation. Ineffective isolation will be noted by florescence appearing in the vasculature and tissue proximal to the cannula site. Although easy to use and inexpensive, the half-life of fluorescein is 15 min. Consequently, this method only provides an initial check for cannula placement and perfusion and is not repeatable.

An uncommon method to determine leakage from a regional to whole body circulation is via a modified dye-dilution method (32). To achieve this, 0.1 mL/kg body weight of 0.5% Evans blue solution is systemically applied at the onset of anesthesia. After 10, 20, and 30 min, the dye concentration in plasma is measured via a spectral photometer at 620 nm. Through extrapolation to zero, the plasma volume and elimination rate of the dye are calculated. After isolation of the extremity, 0.1 mL/kg of the dye solution is injected in the isolated circuit. Plasma dye concentrations in the whole body circulation are measured at 10-min intervals, assessing the presence of a limb to systemic leak. This technique is able to quantify the amount of limb to system leak as a percentage of the volume of the extracorporeal circuit (32). This is a clear-cut advantage over other leak assessment techniques; however, this technique is laborious and does not provide real time assessment.

Continuous monitoring of system leakages requires radio-labeled tracers. Either iodine 131, (^{131}I) technetium 99m (^{99m}Tc)-labeled human serum albumin, or ^{51}Cr-labeled red blood cells are injected into the perfusate (3). The patient is monitored for systemic radioactivity with an isotope scanner placed over the heart or by peripheral blood sampling and gamma counting (37). The use of either albumin or red cells depends on availability. An advantage inherent to using red cells is that this method allows us to quantitate the percentage of drug leaked to the systemic circulation through collaterals in the vascular anatomy. However, unlike albumin, it cannot detect the amount of drug diffused into the soft tissues during perfusion that is then redistributed into systemic circulation (3). Disadvantages of radio-labeled tracers include cost, availability, and the need for cumbersome adjunct equipment (28).

A direct assessment involves assaying melphalan levels from the systemic circulation and the perfusate at regular intervals during perfusion. The samples are immediately cooled, centrifuged, and the serum is stored in liquid nitrogen. A high-performance liquid chromatographic (HPLC) technique is used to assay plasma melphalan concentrations (7). A systemic leak is then defined as systemic melphalan concentration >2% peak melphalan concentration in the perfusate. Obviously this method is quantitative; however, immediate levels cannot be determined, warranting another method of leak detection for use during the procedure.

The physicians at Boston Medical Center have recently reported the use of expired desflurane levels for leak assessment (28). This technique involves administering 3% into the limb circuit by way a vaporizer in line with the gas source. Because desflurane is a volatile anesthetic agent with very low blood gas solubility, relatively small amounts in the blood equate to large volumes in the alveoli. With the addition of desflurane to the limb circuit, (assuming desflurane is not being used as an anesthetic) any expired desflurane detected in the expiratory gases must have originated from the limb, indicating a limb-to-system leak. The respiratory gas monitor has an accuracy of ±0.2% for a range of 0–5%. Thus, the sensitivity of the monitoring combined with the low blood gas coefficient of desflurane makes it ideal for rapid detection in expired gas (28).

Boston Medical Center describes this technique as follows (28). Once limb and systemic hemodynamics are stable, 3% desflurane is introduced into the limb circuit. With good isolation, no desflurane should be detected in the expired breath for a period of 3 min. A limb-to-systemic leak is deliberately induced by partially clamping the venous return to the pump, allowing limb venous pressures to increase above the systemic venous pressure. Within 30 sec, (six breaths of mechanical ventilation), desflurane appeared in the expired breath at a concentration of 0.2%. The nonleak hemodynamics are restored by removal of the clamp, and a steady state was again achieved with no desflurane in the expired breath (28). This leak technique is very quick and relatively simple to perform. However, as of yet, there is no way to quantify the relation between desflurane percentages and systemic melphalan levels.

Other factors indicative of a leak include temp, reservoir levels, ACT, and hematocrit. All of these indices are inherent parts of the procedure already under observation and do not require extraneous equipment or calculations. However, these indices cannot confirm or quantitate the presence of a leak, thus are not primary or sole indicators

of system integrity. Nonetheless, they are convenient trending parameters when used secondarily with other leak assessment methods.

Because the two systems are isolated, and one is undergoing hyperthermia with its own perfusate, temperature variations occur. Such additional measures as thermal insulating pads or sterile heating blankets can be applied to the limb to minimize heat loss. Likewise, cooling blankets can be employed to maintain normothermic body temperatures. Normally, body temperatures should increase slightly during hyperthermia; however, a continuous upward trend in body temp would suggest a systemic leak and corrective action. Lower leg perfusion leaks would note a precipitous rise in bladder temp; whereas, upper limb perfusions leaks increase the nasopharyngeal temperature. However, temperature changes can be a delayed assessment, because a large and potentially dangerous leak may have already occurred before the core temperature changes enough to be detected (28).

During ILP, a dynamic balance exists between the systemic vascular compartment and the vascular compartment of the isolated limb. This relationship is dependent upon the native cardiac output and limb pump flow and central venous pressure and limb venous pressure. Unlike its cardiac application, there is very little manipulation of arterial limb flow once the target flow and temperatures are achieved. Moreover, unlike a cardiac procedure, there is no manipulation at the operative field impairing venous return during perfusion. Thus, once limb and system pressures have equilibrated, monitoring of the reservoir level of the perfusate is useful in attempting to predict and warn of systemic leakage (38).

Some perfusionists find venous reservoir levels are less sensitive regarding leak assessment (7). This may be in part due to venous volume loss over time as a consequence of vasodilatation, increased venous capacitance and probable 3rd spacing. Subtle volume loss is normal and may warrant periodic crystalloid administration during the case. In some instances, difficulty exists trying to determine if the fluid loss is attributable to a leak, or is caused by compartmental fluid shifts in the limb.

Hematocrits and ACTs provide an additional nonspecific index to assess circulatory fluid shifts. In general, the limb hematocrit is lower than the body because of hemodilution. Thus, increasing hematocrits of the limb may indicate a body-to-limb leak, particularly if accompanied by rising venous reservoir levels. A downward trend in the patient's hematocrit may suggest the opposite, a limb-to-system leak, particularly in the presence of falling venous reservoir levels. However, hematocrit will also fluctuate with fluid administration and diuresis, thus it is only a gross indicator of system integrity and should be used as such. Similarly, ACTs of the body and ILP circuit should differ. As with hematocrits, wide shifts between the ACTs of the two systems may alert the clinician to potential leaks, but it cannot confirm or quantify them.

TEMPERATURE MANAGEMENT

Temperature management involves monitoring patient core temps and several limb temps. Generally four or more thermistor probes are placed in the tumor and in the proximal and distal areas of subcutaneous tissue or muscle. Perfusate temperatures are raised to 42°C, with temperatures of the skin and tissue reaching between 38–41°C (1, 32, 37, 39, 40). It has to be borne in mind that the normal temperature within extremity and tumor tissue ranges from 32.5–35°C, far from core temperatures (2). To facilitate limb warming, heating lamps, heated blankets, aluminized plastic sheets, elevated room temps, and sterile water-heated circulating blankets may be used.

Temperature management during ILP is classified into true hyperthermia >41.5°C, mild hyperthermia 38.5–41°C and controlled normothermia 36–39°C (2). The direct relationship of cytostastic drugs efficacy and hyperthermia is well known, (1) with the in vitro cytotoxic action highest at 42°C. In vivo, however, temperatures exceeding 42°C may result in severe tissue necrosis sometimes necessitating limb amputation (5). Although, once tissue temps are lowered to 37–38°C, most perfusion-related complications are eliminated (2).

Nevertheless, increasing the temp to 39–41°C is appropriate as the heat radically improves tissue perfusion, the distribution of the cytostatic agents and tumor response (5). Two separate centers in Rome and Milan showed a significant increase in complete tumor response rate following ILP from 27 to 54%, and 32 to 76% by increasing temperatures from less than 40° to 41.5°C (41, 42). In light of this evidence, standard practice for most institutions is to target tissue temperatures between 38–41°C (2).

CHEMOTHERAPY

Once target temperatures are reached and isolation confirmed, the appropriate chemotherapy agent is given. Since 1957, the agents used for ILP have been alkylating agents. Alkylating agents are dosed by patient weight and work by cross-linking with DNA to block cell division and protein synthesis (1). Original perfusions used nitrogen mustard, HN2, which is a rapidly acting, effective alkylating agent with severe vesicant, (blistering) properties. It was highly effective in inhibiting tumor growth; however, its use increased local toxicity, particularly to peripheral nerves, resulting in neuritis and paralysis (1). Subsequently, other agents were sought having the effectives of nitrogen mustard with lesser toxicity.

Melphalan is and has been the primary drug of choice for ILPs. It is a second-generation alkylating agent that is

a longer acting, mildly vesicant, and soluble in alcohol or propylene glycol (43). Melphalan may be used alone, or in conjunction with other drugs at a lesser dose. These include Thiotepa, Actinomycin D, and Cisplatin (1). Thiotepa is another second-generation alkylating agent. Cisplatin (platinol) is not an alkylating agent, but a platinum-containing molecule used mostly for solid tumors and metastases. Actinomycin D, used to a lesser extent, is an antibiotic interacting with DNA. The decision to use multiple agents is physician preference. Patients having the procedure done for a second or third time will typically receive a combination of drugs versus only melphalan.

MELPHALAN ADMINISTRATION AND DOSAGE

Chemo agents are usually added in multiple doses when the target limb temp is reached, usually 40°C (26). Melphalan is given in 10–20 mg aliquots into the arterial line at 3-min intervals until the entire dose has been delivered (2). It may also be delivered as a single dose in the pump reservoir (1). After administration, limb perfusion is sustained for 60 min. It should be noted that administration of an alkylating agent will increase blood pH in the limb to >7.60.

The melphalan dose is calculated in mg per kg of actual body weight, with a maximum threshold for upper and lower extremities at 80 mg and 100 mg. Although the maximum doses of melphalan are well established, the doses administered during ILP vary according to the extremity of perfused, patient body weight, and overall patient health. Studies at Tulane University School of Medicine found that upper extremities could tolerate a dose of 13 mg/L and lower limbs only 10 mg/L (1). (Isolation is easier in upper extremities versus lower.) Conversely, Boddie and associates use 14 mg/L upper and 16 mg/L for lower because of higher limb volumes in the legs (40, 45). Overall, higher melphalan doses are tolerated if the perfusate is whole blood, the perfusate volume is increased, (1) or if the limb is proportionally larger and more muscular as compared to the rest of the body. Likewise, Melphalan dosage is lowered if other chemotherapy agents are given, as well as in fair skinned, elderly, and chronically ill patients (1).

WASHOUT

After 60 min of perfusion, washout occurs. Washout entails diverting the chemo-enriched perfusate from the circuit and replacing the displaced volume with crystalloid. This is accomplished by the placement of tubing clamps on the venous line redirecting the perfusate into the appropriate waste receptacle. Most centers either rinse with a set amount of volume, ranging from 300–3 L (1, 7, 26, 29, 30, 40) or until the circuit becomes relatively clear and blood free (29, 30). The heater cooler is disconnected from the oxygenator during washout, and the limb passively cools. Notable changes in limb hemodynamics may occur because of the temperature and viscosity changes in the limb at this point in the procedure.

The choice of rinse solution varies between institutions. Some centers use 1000 cc of whole blood (32); however, the majority of institutions wash with the crystalloid solution used in priming or a low molecular weight dextran (1, 7, 26, 40). Many centers use both types of crystalloids. The initial circuit flush occurs with NSS, LR, or plasmalyte until the effluent becomes clear, and then the low molecular weight dextran solution is transfused and remains in the limb (3, 28, 30). The rationales for using a low molecular weight solution are to enhance microcirculation of the limb, lower the embolic risk, and reduce edema (29). Depending on the extent of hemodilution, PRBC may also be transfused through the pump into the limb (3, 40) or given by anesthesia. Upon the completion of the washout phase, tourniquets are released (if used,) cannulas are withdrawn, and vessels repaired.

PATIENT SELECTION CONSIDERATIONS

High-risk patients not eligible for the procedure include those with severe peripheral vascular disease (PVD), lymphedema, peripheral neuropathy, or pregnancy. Regarding age, perfusions are tolerated well by patients in their 70s and 80s (3). Elderly patients meeting the criteria for the procedure are not at greater risk of complications than their younger counterparts (3). The concern in these patients is not age, but the higher probability of other disease factors, such as PVD, diabetes, significant heart disease, prior MI, longer smoking history, and so forth.

COMPLICATIONS

A search was conducted in the English language from 1980 to 1995 reporting ILPs done with melphalan alone or combined with other agents (46). All published series were analyzed for the rate of mortality, number of major amputations and presence of leukopenia. Leukopenia is the principal complication associated with ILP subsequent to the systemic leakage of chemotherapy during perfusion. Overall, leukopenia occurred in 0.7% of patient reviewed. Other nonfatal complications reported involve postprocedure edema 7%, seroma 6%, wound infection/separation 13%, and arterial or venous thrombosis 0.1% (3). Occasionally, temporary loss of nails or sloughing of superficial skin of palms and soles has been seen in addition the cessation of hair growth in the perfused limb for 3 to 6 months (1). The 30-day mortality rate for >2000 patients was 0.6%. Death resulted from cardiopulmonary complications or overwhelming sepsis from the leukopenia. Ma-

jor amputations occurred in 0.8% patients and most were of the lower extremity and occurred before 1966 (46, 1).

FUTURE

The role of the immune system in inducing regression of melanoma has stimulated considerable interest in the use of biologic therapy. These therapies may be alone, as in the case of a vaccine or as an adjunct to existing therapies. In ILP, recombinant tumor necrosis factor alpha, TNF-α, administered with melphalan is undergoing experimental clinical trial in Europe. TNF-α is a biological modifier, given that it is a cytokine derived from activated monocytes and macrophages. It works by inducing hemorrhagic necrosis of tumors in vivo (47). Currently TNF-α is being tested for the treatment of recurrent limb melanomas, in addition to the treatment of stage IV melanomas. Research is in preliminary stages and ongoing.

The theories of isolated hyperthermia perfusion may be suited for other oncology applications. Its use has been documented for treating osteogenic sarcomas in the limb and pulmonary metastases (47, 48). The later therapy involves delivering chemo isolated to one or both lungs, for treating cancers metastasizing from colon carcinoma, melanoma, metastatic sarcoma, renal cell carcinoma, and adenoid cystic carcinoma. Like ILP, it aims to deliver a targeted dose of chemotherapy with minimal systemic toxicity. Both human and animal studies strongly suggest it to be a safe method of treatment; however, more research is required to establish proper regimens, doses, and outcomes (47).

CONCLUSION

It is estimated that 1 in 75 Americans will develop invasive melanoma within the next year (49). Unfortunately this is a conservative approximation, as only about half of all melanomas are actually reported (31). For many years, ILP had limited use. Many practitioners considered the methodology of isolated chemotherapeutic perfusions obscure (50). Moreover, the available data were confusing and many found the technical conduct of the operation overwhelming (51, 52). However, as time goes on, and the behavior of melanoma has become progressively defined, more and more groups now recognize that perfusion chemotherapy is the treatment of choice for recurrent melanoma confined to an extremity, (i.e., satellites, in-transits, positive nodes).

REFERENCES

1. Krementz ET, Sutherland CM, Muchmore JH. Isolated hyperthermia chemotherapy perfusion for limb melanoma. Surg Clin North Am. 1996;76:1313–30.
2. Hohenberger P, Kettelhack C. Clinical management and current research in isolated limb perfusion for sarcoma and melanoma. Oncology. 1998;5:89–102.
3. Ariyan S, Poo WJ. Safety and efficacy of isolated perfusion of extremities for recurrent tumor in elderly patients. Surgery. 1998;123: 335–43.
4. Krementz ET, Knudson L. The effect of increased oxygen tension on the tumorcidal effect of nitrogen mustard. Surgery. 1961;50:266–7.
5. Cavaliere R, Ciocatto EC, Giovanella BC, et al. A selective heat sensitivity of cancer cells: Biochemical and clinical studies. Cancer. 1967;20:1351–81.
6. Rossi CR, Foletto M, Vecchiato, et al. Management of cutaneous melanoma M0: State of the art and trends. Eur J Canc. 1998;33:2320–12.
7. Kam PCA, Thompson JF. Anesthetic experience with isolated limb perfusion with melphalan for melanoma. Perfusion. 1996;11:39–399.
8. U.S. Department of Food and Drug Administration. Office of Orphan Products Development. Rockville, MD; 1992. check f27.
9. American Committee on Cancer. AJCC Cancer staging manual. Malignant melanoma of the skin. Philadelphia: Lippencott-Raven, 1997: 163–70.
10. Nagle RB. The use of antikeartin antibodies in the diagnosis of human neoplasma. Am J Clin Pathol. 1983;79:458.
11. Asada M. Solid basal cell epitheliomas possibly originates from the outer root sheath of the hair follicle. Acta Derm Venereol. (Stockh). 1993;73:286.
12. D'Errico M, Dogliotti E. The role of p53 mutations in skin cancer. Chron Dermatol. (Roma). 1996;6:27.
13. Spencer JM. Activated rat genes occur in human actinic keratoses, premalignant precursors to squamous cell carcinomas. Arch Dermatol. 1995;131:792.
14. Miller DL, Weinstock MA. Nonmelanoma skin cancer in the United States: Incidence. J Am Acad Dermatol. 1994;30:774.
15. Langely RGB, Barnhill RL, Mihm MG, et al. Neoplasms: Cutaneous melanoma. In: Clinical Dermatology. Freedberg IM, Eisen AZ, Wolff K, eds. New York: McGraw-Hill, 1999;1080–116.
16. Rhodes AR, et al. Risk factors for cutaneous melanoma: A practical method for recognizing predisposed individuals. JAMA. 1987;258: 3146.
17. Elwood JM. Melanoma and sun exposure. Semin Oncol. 1996;23:650.
18. Pappa AS, et al. Childhood melanoma. In: Cutaneous Melanoma. Balch CM, ed. St Louis: Quality Medical, 1998;175–86.
19. Ceballus PI, et al. Melanoma in children. N Engl J Med. 1995;332: 656.
20. Koh HK, et al. Evaluation of the American Academy of Dermatology's national skin cancer early detection and screening program. J Am Acad Dermatol. 1996;34.
21. Friedman RJ, Rigel SD, Kopf AW. Early detection of malignant melanoma: the role of physician examination and self-examination of the skin. CA Cancer J Clin. 1985;35:140–51.
22. Anderson B, Ostberg J. Long-term prognosis in geriatric surgery. J Am Geriatr Soc. 1972;20:255.
23. Clark WH, From L, Bernadino EA, et al. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. 1969;29:70.
24. Breslow K. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg. 1970;172:902.
25. Jacques DP, Coit DG, Brennan MF. Major amputation for advanced melanoma. Surg Gynecol Obstet. 1989;169:1–6.
26. Hafstrom L, Rudenstam CM, Blomquist E, et al. Regional hyperthermic perfusion with melphalan after surgery for recurrent malignant melanoma of the extremities. J Clin Oncol. 1991;9:2091–4.
27. Klaase JM, Kroon BB, van Geel AN, et al. Prognostic factors for tumor response and limb recurrence free interval in patients with advanced melanoma of the limbs treated with regional isolated perfusion with melphalan. Surgery. 1994;115:39–44.
28. Stanley G, Sundarakumara R, Crowley R, et al. Desflurane as a marker of limb to systemic leak during hyperthermic isolated limb perfusion. Anesth. 2000;93:574–6.
29. Fried SJ, Miller R, Weaver F. Safe, compact, and portable system for regional chemotherapeutic hyperthermic perfusion procedures. J Extra-Corp Technol. 1993;25:22–6.

30. Pfefferkorn RO, Didolkar MS. Regional perfusion for melanoma of the extremities. J ExtraCorp Technol. 1982;3:475–9.
31. Polk HC. Surgical progress and understanding in the treatment of melanoma epidemic. Am J Surg 1999;178:445–8.
32. Ghussen F, Nagel K, Groth W, et al. A prospective randomized study of regional extremity perfusion in patients with malignant melanoma. Ann Surg. 1984;200:764–8.
33. DeBois W, Sukhram Y, McVey J, et al. Reduction in homologous blood transfusions using a low prime circuit. J Extra-Corp Technol. 1996;28:58–60.
34. O Gara P, Treanor P, Lilly K, et al. Technique for routine use of heparin-bonded circuits with a reduced anticoagulation protocol. J Extra-Corp Technol. 2000;4:207–13.
35. von Segesser LK, Weiss BK, Pasic M, et al. Risks and benefits of lower systemic heparinization during open-heart operations. Ann Thorac Surg. 1999;58:391–8.
36. Aldea GS, Doursounian M, O'Gara P, et al. Heparin-bonded circuits with a reduced anticoagulation protocol in primary CABG: A prospective randomized study. Ann Thorac Surg. 1996;62:410–8.
37. Koops HS, Vaglini M, Suciu S, et al. Prophylactic isolated limb perfusion for localized, high-risk limb melanoma: Results of a multicenter randomized phase III trial. J Clin Oncol. 1998;16:2906–12.
38. Crutchely OM, Kaplan JA, Waller JL, et al. Anesthesia for isolated hyperthermic limb perfusion. Anesthesiology. 1982;57:228–30.
39. Thomspon JF, Hunt JA, Shannon KF, et al. Frequency and duration of remission after isolated limb perfusion for melanoma. Arch Surg. 1997;132:904–7.
40. Edwards MJ, Soong SJ, Boddie AW, et al. Isolated limb perfusion for localized melanoma of the extremity. Arch Surg. 1990;125:317–22.
41. Cavaliere R, Cavaliere F, Deraco M, et al. Hyperthermic antiblastic perfusion in the treatment of stage IIIA-IIIAB melanoma patients: Comparison of two experiences. Melanoma Res. 1994;4(Suppl 1):5–11.
42. DiFilippo FV, Calabro A, Ciannarelli D, et al. Prognostic variables in recurrent limb melanoma treated with hyperthermic antiblastic perfusion. Cancer. 1989;63:2551–61.
43. Chabner BA, Wilson W. Pharmacology and toxicity of antineoplastic drugs. In: Williams Hematology. Beutler E, ed. New York: McGraw-Hill, 1995;143–55.
44. Kopf AW, et al. Thickness of malignant melanoma: Global analysis of related factors. J Dermatol Surg Oncol. 1987;13:345.
45. Boddie AW, Briele H, Krementz E, et al. A phase I study of melphalan in 40°C isolated limb perfusion using packed red blood cells and lactated Ringer's perfusate. Proc Am Soc Clini Oncol. 1992;11: A1208.
46. Taber SW, Polk HC. Mortality, major amputation rates, and leukopenia after isolated limb perfusion with phenylalanine mustard for the treatment of melanoma. Ann Surg Oncol. 1997;5:440–5.
47. Weksler B, Burt M. Isolated lung perfusion with antineoplastic agents for pulmonary metastases. Chest Surg Clin North Am. 1998; 8:157–82.
48. Vaglini M, Bacci G, Baldini M. Limb salvage in osteogenic sarcoma of extremities a new therapeutic approach associated infusion and hyperthermic antiblastic perfusion. J Extra-Corp Technol. 1987;19: 338–47.
49. Cosary CL, et al. SEET cancer statistics review, 1973–1992: National Cancer Institutes. NRH Pub 96-2789, Bethesda, MD, 1995.
50. Creech O, Krementz ET, Ryan RF, et al. Chemotherapy of cancer: Regional perfusion utilizing and extracorporeal circuit. Ann Surg. 1958;148:616–32.
51. Krementz ET, Ryan RF, Carter RD, et al. Hyperthermic regional perfusion for melanoma of the limbs. In: Cutaneous Melanoma: Clinical Management and Treatment Results Worldwide. Balch CM, Milton GW, eds. Philadelphia: Lippincott, 1985:171–95.
52. Schwemmle K, Aigner K, eds. Vascular Perfusion in Cancer. New York: Springer, 1083.