RÉSUMÉ
Traitement des récidives locales et en transit des mélanomes par perfusion de membre isolé : intérêt de l’échographie Doppler

But. Déterminer l’intérêt de l’échographie-Doppler couleur (EDC) pour évaluer l’évolution des récidives locales et/ou en transit de mélanome, traitées par chimiothérapie en perfusion de membre isolé (MIP).

Matériels et méthodes. Analyse rétrospective, chez 18 patients traités par membre isolé perfusé, des résultats des échographies-Doppler couleur pré et post traitement, étudiant le nombre, la taille et la vascularisation des cibles tumorales. Les résultats ont été comparés aux données cliniques et à l’évolution.

Résultats. L’échographie a permis une évaluation plus précise que la clinique en ce qui concerne le nombre et la taille des nodules de récidive locale et des métastases en transit. La diminution de volume et la disparition précoce de la vascularisation intranodulaire étaient associées à une bonne réponse au traitement. La rémission complète a été d’autant plus précoce que la disparition de la vascularisation était précoce. La présence initiale ou la persistance dans les 3 mois d’une vascularisation tumorale ne sont pas apparues comme des critères pronostiques défavorables. L’apparition d’une vascularisation semblait traduire prématurément l’échec du traitement.

Conclusion. L’échographie de haute fréquence est un examen précis pour l’évaluation et la surveillance des patients suivis pour mélanome récurrent des membres. Elle fournit des éléments pronostiques permettant de discuter très tôt une modification de la stratégie thérapeutique.


ABSTRACT
Purpose. To assess the effectiveness of Color Doppler Ultrasound (US) in the follow-up of recurrent melanoma of the limbs treated by isolated limb perfusion (ILP).

Materials and methods. A retrospective study was done looking at 18 patients treated by isolated perfusion of the limb. Number, size and vascularity of subcutaneous metastases were examined for all patients. The results of pre and post therapeutic examinations were compared with clinical data and evolution.

Results. US was superior to clinical examination in the detection and measurement of subcutaneous metastases. Decrease in tumor size and early disappearance of tumoral vascularity were associated with an effective tumor response to treatment. Early disappearance of vascularity was associated with early complete response. Initial presence or persistence of vascularly before the third month after treatment were not poor prognostic factors. The appearance of vascularity seemed to be an indicator of early failure of the treatment.

Conclusion. Color Doppler US is highly effective for the follow-up of patients with recurrent melanoma of the limbs. It provides prognostic factors of treatment failure, which may lead to early modification of the treatment strategy.

in the evaluation of patients with recurrent limb melanoma treated with ILP using Melphalan between May 1999 and October 2001.

**Patients**

The following inclusion criteria were used: the patients must have had prior surgery for limb melanoma with histological confirmation of diagnosis with local recurrence and/or in-transit metastatic nodules for which ILP had been performed, irrespective of previous therapy. Patients must have had pre-ILP baseline CDUS and at least one follow-up CDUS examination within 3 months post-ILP. Patients must have had clinical follow-up of more than six months. Twenty-two patients underwent ILP for recurrent limb melanoma between May 1999 and October 2001. One patient was excluded because pre-ILP baseline CDUS was not available, and three patients were excluded because clinical follow-up was less than six months. A total of 18 patients were included (9 males and 9 females), with a mean age of 57 years (range: 27-75). For one patient, this was the second ILP treatment.

**Target lesions**

The lower limb was involved in 17 cases, and the upper limb in 1 case. Lesions were either local recurrences (within 5cm of excision scar from primary lesion) or in-transit metastases (non-nodal metastases >5cm from scar and proximal to the first regional nodal drainage station). All lesions were superficial, cutaneous or subcutaneous, allowing high-frequency high-resolution CDUS evaluation, even in the presence of diffuse edema post-ILP. In addition to these target lesions, regional nodal metastases were present in 9 cases.

**Materials**

A PowerVision 8000 digital US unit (Toshiba, Japan) equipped with a 12 MHz transducer was used. This US unit allows storage of color-coded images directly into a PC or PACS system. Technical parameters were preset for detection of slow flow: PRF <1500Hz, low frame rate. Because of the large number of target lesions, contrast agents were not used.

**Methods**

For each patient and at each evaluation, the number of lesions was recorded and lesions were measured. When lesions were numerous, those that were better visualized, better defined and more easily anatomically detectable were selected as target lesions and recorded on a diagram. The 3 largest perpendicular diameters of each target lesion were measured in mm using electronic calipers. Tumor vascularity was evaluated using CDUS. The technical parameters were preset to optimize detection of slow flow based on previous publications on flow evaluation of melanoma-type skin tumors (9, 10). Each tumor focus was scanned along its long axis to ensure imaging of the entire lesion. Because most lesions were small, intra-tumor vascularity was used in only 5% of cases. This technique may prevent or delay amputation but global survival rate has not increased. While several prognostic factors have been evaluated to predict tumor response (2-4, 7), clinical evaluation, based on measurement of target lesions, remains the standard of reference for its evaluation. Since 1999, we have used high frequency US to detect and measure in-transit cutaneous and subcutaneous metastases from melanoma. Using color Doppler US (CDUS) with high frequency transducers, we have reported that flow could be detected in intra-tumor vessels larger than 100 microns from xenografted superficial tumors into mice (8). As part of a protocol of ILP with Melphalan, we wished to assess the usefulness of CDUS for the evaluation of number and size of target lesions prior to ILP and evaluation of objective response to therapy. We wished to determine if the presence of intra-tumor vessels, their disappearance or appearance after treatment were prognostic factors. We report the preliminary results from a retrospective study on a relatively limited number of patients; a prospective study on a larger patient population with longer-term follow-up is currently under way.

**MATERIALS AND METHODS**

We have retrospectively compared the results of clinical evaluation and CDUS imaging of the entire lesion. Because of the large number of target lesions, contrast agents were not used.

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vascularity was recorded as present or absent, without further quantitative or semi-quantitative analysis. Each patient underwent baseline pre-ILP CDUS. Baseline CDUS evaluation of intra-tumor flow was performed in 16 of 18 patients. Post-ILP follow-up CDUS was performed between 8 to 23 days in 15 patients and between 6 weeks to 3 months in 3 patients. Lesion size and vascularity were evaluated and compared to the baseline scan. Two experienced sonographers with expertise in the evaluation of superficial and cutaneous tumors performed all CDUS examinations, and each patient was scanned by the same sonographer prior to and following ILP. Results were correlated with response type as determined at follow-up clinical and CDUS evaluation: complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD).

Isolated limb perfusion protocol

The absence of atheromatous plaques and deep venous thrombosis was confirmed at pre-ILP CDUS. The dose of Melphalan was calculated based on limb volume, with a maximum of 70mg for an upper limb and 140mg for a lower limb. The procedure was performed under general anesthesia. The arterial and venous catheters were placed according to lesion location: iliac or femoral for the lower limb and axillary or subclavian for the upper limb. A surgical cut down of the target vessels was performed for cannulation and all potential collateral vessels were tied down. A tourniquet was placed proximally on the limb and the extracorporeal circulation started using a solution of 500cc of Ringer’s lactate with 2500 units heparin. The perfusion rate varied from 300 to 500cc/min. A heat exchanger was added to the perfusion circuit to achieve a tissue temperature of 38-40 degrees Celsius. Temperature monitoring was achieved with thermal sensors placed at the proximal and distal ends of the limb with continuous computerized temperature recording.

Leakage into the systemic circulation was monitored by injecting Tc99m-tagged RBC (200 microCu) into the perfusion circuit and placing a camera over the patient’s chest. The maximum allowable leakage was 10%. After a tissue temperature of 39 degrees Celsius and hemodynamic equilibrium were reached, Melphalan was directly injected into the arterial line of the perfusion circuit. The limb was perfused for 90 minutes. The chemotherapy perfusate was then chased with Ringer’s lactate until the venous line appeared clear. The tourniquet was released. Arterial and venous cannulas were removed and the cut down was sutured. The heparin effect was reversed with protamine. The first post-ILP night was spent in the post-anesthesia care unit. Regional side effects usually developed the following day and included edema, erythema, and sometimes blistering. These usually resolved within 3 to 6 months but longer-term complications including ankle stiffness, skin discoloration, and cutaneous sclerosis could persist. These complications could preclude adequate clinical evaluation of objective response to treatment (11).

RESULTS

Correlation between clinical and CDUS findings at baseline evaluation

The number of clinically detected metastatic lesions was recorded in 9 of 18 patients, and ranged from one to “about twenty”. For the other patients, recorded descriptions included induration near a scar from previous surgery, multiple non-palpable lesions or recurrences, and numerous nodules without specification of the exact number. At CDUS, 130 nodules were detected in 16 of 18 patients, ranging from 2 to 25 per patient. In 2 patients, a hypoechogenic infiltrative process without discrete nodule was noted. For the 9 cases where the number of clinically detected lesions was specified, CDUS showed additional lesions in 6 cases (fig. 1) and the same number of lesions in 1 case. For the remaining 2 cases, the clinical notes described “about 10” lesions in one case and “about 20” lesions in the other case whereas CDUS detected 3 and 7 lesions respectively. Clinical measurements were recorded for only 15 lesions in 8 patients, with lesion sizes ranging between 5 and 80mm. A total of 110 nodules in 16 patients were measured at US, with lesion sizes ranging between 2 and 55mm. When possible, correlation between clinical and US measurements showed that lesion measurements were larger at clinical evaluation compared to US evaluation.

At baseline CDUS, lesions were evaluated for the presence of intra-tumor vessels in 16 of 18 patients. For 12 patients, there was at least one visible intra-tumor vessel in at least one lesion (fig. 2), while no intra-tumor vessel was detected for the remaining 4 patients.

Correlation between objective tumor response at 6 months and post-treatment CDUS findings

Early results for size and vascularity of target lesions are reported in table I. Treatment efficacy was manifested by rapid size reduction of lesions in 12 of 18 patients and by persistent absence of intra-tumor vessels (n=4) or disappearance of intra-tumor vessels seen at baseline CDUS (n=7). Size reduction and vessel disappearance occurred in combination for 5 patients. Objective response at 6 months based on lesion size evolution compared to the post-ILP CDUS is reported in table II. Of the 12 patients where initial post-ILP CDUS showed size reduction of lesions, 6 were in CR and 6 were in PR (fig. 3) at 6 months. Three of 5 patients with stable lesions at CDUS within the first 3 weeks post-ILP had progressive disease at 6 months. One patient showed early post-ILP progression of disease. Objective response at 6 months based on intra-tumor vascularity evolution on the post-ILP CDUS is reported in table III. Of the 4 patients with persistent absence of intra-tumor vessels and the 7 patients with disappearance of intra-tumor vessels after treatment, 4 were in CR and 5 were in PR at 6 months; 2 had PD with appearance or re-appearance of intra-tumor vessels (fig. 4) at CDUS within target lesions. Overall at 6 months, of the 14 patients with CR (n=6), PR (n=7) or SD (n=1), 9 (64%) had lesions with intra-tumor vessels at baseline CDUS. Intra-tumor vessels had disappeared at early post-ILP CDUS in 6 cases (67%) after a mean of 5 weeks post-ILP (range: 1 week – 2 months). Three (75%) of the 4 patients with PD had lesions with intra-tumor vessels at baseline CDUS. At the time of objective response assessment, all lesions had intra-tumor vessels.

Final clinical and CDUS assessment of objective response post-ILP (table IV)

CR was achieved at a mean of 4.3 months (range: 1-15) in 8 cases (44.4%). Five of these had relapse of disease after a mean CR period of 7.8 months (range: 1-23). Three had persistent CR at 3, 4 and 13 months respectively. For these 8 patients, lesions had decreased in size at the first post-ILP evaluation. Seven had had pre-ILP baseline CDUS that showed absence of intra-tumor vessels in 2 and presence of intra-tumor vessels in 5 that had disappeared at a mean of 2.2 months (range: 1 week-6 months) post-ILP. The earliest
CR was at 1 month and for the patient with lesions that showed the fastest disappearance of intra-tumor vessels. The patient where intra-tumor vessel regression was the slowest (6 months) is the one that achieved CR the latest (at 15 months).

PR was achieved in 5 cases (27.7%). In 4 cases, PR had a mean duration of 8 months (range: 5-16) prior to disease progression. The remaining patient had persistent PR at the last follow-up visit. The first post-ILP CDUS evaluation in these 5 patients had shown lesion size reduction in four and stable lesion size in one. Three patients had lesions with intra-tumor vessels at baseline pre-ILP CDUS that disappeared in 2 cases post-ILP, at 1 month and 8 days respectively. For the remaining 2 patients, intra-tumor vascularity was absent at baseline pre-ILP CDUS and remained absent at post-ILP CDUS. One patient (5%) had stable disease, without significant lesion size change post-ILP and persistent intra-tumor vessels at CDUS. Four patients (22.2%) showed PD. None of these patients had shown lesion size reduction on the first post-ILP CDUS and intra-tumor vascularity had either persisted or appeared or re-appeared secondarily.

**DISCUSSION**

These preliminary results show the usefulness of high frequency color Doppler US imaging for the evaluation of local recurrence and in-transit subcutaneous metastases in patients with melanoma of a limb.

Pre-treatment CDUS provides precise evaluation of target lesions. CDUS allows detection of nodules that are too small or too deep to be clinically detectable and identification of discrete target nodules at sites where clinical evaluation only shows induration or infiltration. The US appearance of these conspicuous hypoechoic nodules, even as small as a few mm in size, is characteristic. The superior efficacy of US compared to clinical examination for the evaluation of number and size of subcutaneous and nodal metastases from melanoma has already been reported in two publications (14, 15). Both of these studies were performed in a different clinical setting where US examinations were performed at the time of follow-up of patients after surgical resection of a primary melanoma in order to detect metastatic disease, mainly regional nodal metastases. However, the study by Blum et al. (15) reported that US detected in-transit metastases that were not clinically palpable in 4 of 24 patients (16.7%). Following initial treatment, US is an important modality for the assessment of objective response, especially to differentiate between CR and PR. In 5 cases from our series, clinical examination revealed no residual palpable nodule, suggesting CR, whereas US

**Table I:**
Results of the first US and Doppler studies after treatment. Evolution of size and vascularity of tumor targets compared with baseline Doppler US results.

<table>
<thead>
<tr>
<th>Vascularity</th>
<th>Decreased n=12</th>
<th>Stable n=5</th>
<th>Increased n=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disappearance</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Persistence</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Appearance</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Absent</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Not evaluated</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table II:**
Objective response before 6 months, function of tumor size evolution on the first US study after treatment.

<table>
<thead>
<tr>
<th>Objective response</th>
<th>Decreased n=12</th>
<th>Stable n=5</th>
<th>Increased n=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>PR</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PD</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table III:**
Objective response before 6 months, function of tumor vascularity evolution on the first Doppler study after treatment.

<table>
<thead>
<tr>
<th>Objective vascularity</th>
<th>Disappearance n=7</th>
<th>Persistence n=5</th>
<th>Absent n=4</th>
<th>Not evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>PR</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PD</td>
<td>1*</td>
<td>2</td>
<td>1**</td>
<td></td>
</tr>
</tbody>
</table>

* intra-tumor vessels had disappeared at early post-ILP evaluation but later re-appeared at the time of PD. ** intra-tumor vessels secondarily appeared at the time of PD.
Table IV:
Evaluation of response to treatment with ILP.

<table>
<thead>
<tr>
<th>Objective response</th>
<th>At 6 months</th>
<th>Final evaluation</th>
<th>Evaluation of vascularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>6</td>
<td>8 (44.4%)</td>
<td>Presence of vascularity in 5/8 at baseline CDUS; Disappearance=100%</td>
</tr>
<tr>
<td>PR</td>
<td>7</td>
<td>5 (27.7%)</td>
<td>Present in 3/5; Disappearance in 2/3 (66%); Persistence in 1/3 (33%); Persistent vascularity</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>1 (5.5%)</td>
<td>Persistent in 2/4 (50%); Appearance in 1/4 (25%); Re-appearance in 1/4 (25%)</td>
</tr>
<tr>
<td>PD</td>
<td>4</td>
<td>4 (22.2%)</td>
<td>Persistence in 1/3 (33%)</td>
</tr>
</tbody>
</table>

showed residual nodules, even though smaller than pre-ILP, indicating PR. Clinical measurement of lesions is difficult. Either no nodule is palpable or small nodularities are palpable near scars and grafts. Larger lesions often are associated with an inflammatory reaction and depth extension cannot be appreciated. At US, even in the presence of induration or infiltration, lesions most often are easily detectable, hypoechoic, and nodular with sharp margins. Lesion measurements can easily and accurately be obtained using electronic calipers. In this study, we measured all target lesions in 3 planes, but analysis of data showed that the accumulation of measurements complicated the evaluation without increasing the precision. The role of US for the evaluation of cutaneous and subcutaneous lesions is dual. First, after all lesions have been detected, to select a maximum of 5 target lesions per patient, ideally with a minimum of 10 mm in diameter each, that are easily visible, accessible and measurable and precisely localize them using a diagram for reproducibility at follow-up. Second, to obtain measurements for these lesions that are as precise as possible using the RECIST (Response Evaluation Criteria in Solid Tumors) guidelines, i.e. measurement of the longest diameter per lesion (12, 13).

Several prognostic factors have been identified for recurrent limb melanoma following ILP, the main ones including the stage of disease, the presence or absence of regional nodal disease, and the number of in-transit metastases. We wanted to assess if the evolution of CDUS findings within the first weeks post-ILP when compared to findings at baseline pre-ILP CDUS had any prognostic value for predicting objective tumor response. Following ILP, clinical examination alone cannot always evaluate early response to treatment because edema and inflammatory change interfere with detection and measurement of lesions, especially the smaller ones. US can always be performed. Our results indicate that US findings between 8 to 21 days post-ILP have some predictive value on outcome, though the extent of which remains to be further determined from a larger scale study. Indeed, the 12 patients where post-ILP CDUS showed decreased lesion size went on to CR or PR whereas 3 of 5 patients where CDUS showed stable lesion size went on to PD. CDUS evaluation of intra-tumor vessels also seems to provide prognostic information. The presence of intra-tumor vessels at baseline pre-ILP evaluation does not seem to correlate with poor treatment response. Indeed, 5 of 12 patients with intra-tumor vessels at baseline CDUS went on to CR and 3 went on to PR. The absence of intra-tumor vessels at baseline CDUS resulted in CR in 2 of 4 cases and PR in 1 of 4 cases. The disappearance of intra-tumor vessels at early post-ILP CDUS seems to be a prognostic factor of treatment response (CR+PR) since 85% of patients (6 of 7) where this was observed went on to CR or PR. Patients (n=8) where early post-ILP CDUS showed size reduction of lesions and disappearance or absence of intra-tumor vessels either had CR (n=6) or PR (n=2). Complete response was recorded earlier in patients with earlier disappearance of intra-tumor vessels.

The persistence of intra-tumor vessels within 3 months post-ILP was noted in 2 of 4 patients with PD but also in one patient with late CR. This criterion alone does not seem reliable to predict poor response to treatment. On the other hand, early post-treatment appearance of intra-tumor vessels seems to be an early indicator of treatment failure. The identification of criteria enabling early detection of poor response to treatment would be helpful to allow early modification of the treatment regimen or management strategy and increase the chances of preventing PD. In the absence of distant metastases, a second course of ILP could be proposed either with Melphalan alone since the efficacy of repeat treatment with this agent has been reported (16) or the combination of Melphalan, TNF-alpha and interferon-gamma (possible in France for patients of melanoma only) is the setting of a research protocol(17-19). Systemic chemotherapy, surgery and carbon-dioxide laser (20) are alternative treatment options when repeat ILP is contra-indicated.

The results of ILP in our 18 patients with regards to CR (44.4%) and PR (27.7%) are similar to results from in the literature (2-6).

The number of patients in this study is limited and conclusive statistical analysis of these preliminary results is not possible. A larger prospective trial using a strict methodology for the selection of target lesions, comparison with clinical data, tumor measurements and quantification of intra-tumor vascularity is currently underway. The use of US contrast agents should improve the sensitivity to identify patients with early response to treatment and those at increased risk of disease progression, especially with the use of flow quantification and analysis software.

Results from this retrospective study of 18 patients show that CDUS allows accurate baseline evaluation of metastatic cutaneous and subcutaneous lesions from melanoma. US detects more nodule than clinical evaluation, allows selection of target lesions, and provides precise and reproducible measurements of the longest diameter of lesions. CDUS allows evaluation of intra-tumor vascularity. Early disappearance of intra-tumor vascularity appears to be a prognostic factor for favorable response to treatment whereas secondary appearance of intra-tumor vascularity seems to be an early prognostic factor for treatment failure. Such information may have significant impact on the management strategy. Histological confirmation of response to ILP treatment is not readily possible and clinical evaluation of treatment efficacy is difficult. Color Doppler US is well suited for the detection, measurement, and evaluation of vascularity of local recurrences and in-transit metastases from melanoma.
Fig. 3: Follow-up of a subcutaneous metastasis treated by ILP. 

a) Grey scale ultrasound 8 days after treatment shows a 24.8×22.3 mm hypoechoic nodule in the subcutaneous tissue. 
b) Ultrasound 23 days after treatment shows a decrease in tumor size: 20.0×16.7 mm. 
c) Three months after treatment: the tumor has continued to decrease in size; it measures 13.8×8.9 mm. The patient is in partial remission.

Fig. 3 : Échographies d’évaluation après traitement par MIP. 

a) J8 après traitement : un nodule sous cutané hypoéchogène mesure 24.8×22.3 mm. 
b) À J23 : le nodule a diminué de volume, il mesure 20.0×16.7 mm. 
c) 3 mois après traitement : le nodule a encore diminué, il mesure 13.8×8.9 mm. Rémission partielle.

Fig. 4: Color Doppler US before and after treatment by ILP. 

a) Color Doppler US before treatment shows a 25 mm cutaneous nodule with numerous intratumoral vessels. 
b) Color Doppler US 7 days after treatment shows a decrease in tumor size and intratumoral vascularity. 
c) Color Doppler US 6 months after treatment: recurrence with increase in tumor size and perfusion.

Fig. 4 : Échographies-Doppler d’évaluation avant et après traitement par MIP. 

a) Avant traitement : gros nodule cutané hypoéchogène avec une dizaine de signaux couleur traduisant la vascularisation tumorale. 
b) À J7 après traitement, le nodule a diminué de volume et la vascularisation intra-tumorale a pratiquement disparu. 
c) 6 mois après traitement : reprise évolutive avec augmentation de volume et réapparition de vaisseaux tumoraux.
Références


