Chirurgia (2010) 105: 637-643
Nr. 5, Septembrie - Octombrie
Copyright© Celsius

Diagnosis and treatment protocols of cutaneous melanoma: latest approach 2010

I. Petrescu[1], C. Condrea[1], A. Alexandru[1], D. Dumitrescu[1], G. Simion[2], E. Severin[3], C. Albu[3], D. Albu[3]

[1]Department of Plastic Surgery and Reconstructive Microsurgery, University Emergency Hospital Bucharest
[2]Department of Pathology, University Emergency Hospital Bucharest
[3]Department of Genetics, "Carol Davila" University of Medicine and Pharmacy

Rezumat

Protocoale de diagnostic și tratament ale melanomului cutanat: actualități 2010

Melanomul cutanat este cea mai agresivă neoplazie cutanată, a cărei incidență continuă să crească în ultimile decenii. Noile tehnici imagistice joacă un rol important în managementul melanomului: dermatoscopia și dermatoscopia digitală, ecografia, RMN, TC, PET și PET/CT. Datorită dermatoscopiei care permite diagnosticarea melanomului în faze incipiente, numărul cazurilor de melanom vindecate a crescut. Biopsia limfonodulului santinelă a devenit o etapă obligatorie pentru tumorile cu grosimea >1 mm. Dozarea biomarkerilor tumorali s-a dovedit o investigație necesară pentru diagosticul, monitorizarea postoperatorie tardivă și răspunsul terapeutic al melanomului. Stadializarea TNM actuală are la bază clasificarea AJCC din 2001 și include noi elemente: ulcerația (determinată histopatologic) în stadiile I și II precum și micro- și macrometastazele ganglionare în stadiul III. Protocoalele terapeutice includ excizia tumorii cu o limită de 1-2 cm precum și limfadenectomia radicală în cazurile în care biopsia limfonodulului santinelă este pozitivă. Tratamentul adjuvant din stadiile avansate incluzând chimioterapia, imunoterapia nespecifică și interferonul a oferit rezultate modeste privind recidiva tumorală și rata de supraviețuire. Metodele terapeutice recente precum nanosferele de aur sau terapia genica sunt în curs de perfecționare și reprezintă alternative terapeutice de viitor. Protocoalele de control incluzând evaluarea clinică, serologică și imagistică au un rol major în detectarea recidivelor tumorale. În pofida noilor informații și al suportului tehnologic, managementul melanomului în stadii avansate rămâne o problemă nerezolvată.

Cuvinte cheie: melanomul cutanat, dermatoscopia, biopsia limfonodulului santinelă, stadialializarea TNM a melanomului, diagnosticul imagistic al melanomului, biomarkeri tumorali, tratamentul chirurgical al melanomului, protocoale de urmărire postoperatorie

Abstract

Cutaneous melanoma is the most aggressive skin malignancies with increasing rate of incidence in the latest decades. New imaging technique plays an important role in melanoma management: dermoscopy and computer dermoscopy, ultrasound, MRI, CT, PET and PET/CT. Due to the dermoscopy and lesion diagnosis in early stages the increasing number of curative melanoma are registered. Sentinel lymph node biopsy became a compulsory phase for patients with tumor thickness >1 mm. Serological biomarkers proved to be a necessary investigation for melanoma diagnosis, follow-up and treatment response. Current TNM melanoma staging is based on AJCC classification since 2001 witch includes new elements like histopathologic ulceration in stage I and II and lymph node micro- and macrometastases in stage III. Treatment protocols include surgical tumor excision with only 1-2 cm safety margins and radical lymphadenectomy is performed after positive sentinel lymph node biopsy. The adjuvant treatment in advanced stages including chemotherapy, unspecific immunotherapy and interferon offers poor results regarding free disease terms rate of survival. The advanced therapeutic procedure like golden nanospheres and gene therapy are recently

Corresponding author: Ina Petrescu, MD
University Emergency Hospital Bucharest
Splaiul Independenței nr. 169, sector 5,
050098 Bucharest, Romania
E-mail: inapetrescu@yahoo.com

studied and represent an alternative for future treatment of melanoma. Follow-up protocols have a great importance for detection of the melanoma recurrences and include clinical, serological and imaging evaluation. Despite all new knowledge and technological support the advanced stage melanoma management still remain an unsolved problem.

Key words: cutaneous melanoma, dermoscopy, sentinel lymph node biopsy, TNM melanoma staging, melanoma imaging diagnosis, serological biomarkers, surgical treatment of melanoma, melanoma follow-up protocols

Introduction

Even in the last decades the incidence of melanoma progressively increased, the mortality remained about at the same level and this thanks to the new diagnosis method like dermoscopy, sentinel lymph node biopsy and appropriate surgical treatment.

Detection melanoma in early stages is the key in disease management, to prevent tumor progression and to obtain curative results.

Epidemiology of melanoma

According to the American Cancer Society, melanoma accounts for about 4 percent of skin cancer cases and is responsible for 90% of skin cancer deaths.The incidence have been rising in past 30 years (1975-2005): from 8 to 22 cases/ 100 000/ year in males and from 7 to 15 cases/100 000/year in females, - numeric values for United States.

Dates available for Romania regards mortality from skin cancer in men and women age 20-44 years in periods 1985-1989 and 1995-1999. Favorable trends was observed for male: 0,87 to 0,85 and downward trends for women :0,67 to 0,84. At the age 45 to 64 years it records unfavorable trends both in male: 4,18 to 5,02 and women: 2,29 to 2,73. (1)

Risk factors for melanoma

The main factor proved to melanoma development is the large numbers of melanocytic nevi at Caucasian race: risk of 10-fold to people with 100 nevi compared to the 1-fold to individuals with 0-10 nevi.

The second factor is the presence of atypical nevi witch implies presence of 3 from 5 clinical aspects: diameter >5 mm, irregular border, ill-defined border, different color and the macular component. A person with about 5 atypical nevi has a risk of 5-fold. In cases with >100 nevi in witch >5 atypical nevi there is a high risk of melanoma of 50-fold.

Also melanocytic nevi are develop during childhood and adolescence when sun exposure is an important factor for its development. Sun burns at this ages but even moderate sun exposure proved to have same influence.

The recent studies have demonstrated that sunscreen alone does not assure protection and clothes are necessary.

Genetic factor is represent by the number of melanocytic nevi both mother and father witch is associated with presence at their children.

Clinical diagnosis

Cutaneous melanoma has a few clinical subtypes: superficial spreading melanoma lentigo maligna melanoma, nodular melanoma, acral lentiginous melanoma, mucosal lentiginous melanoma, amelanotic melanoma. (2)

Superficial spreading melanoma, the most frequent form, accounts 70% of total melanomas. Anatomic localization is on the trunk in man and lower extremities in women. Clinically it presents an irregular and different color (dark-brown, red, white, black) macula. *(Fig. 1)*

Nodular melanoma represents 15%of melanomas and is generally localized on the trunk. It resemble the blue-black nodule witch can be ulcerated.

Lentigo maligna melanoma accounts for 5% of melanoma and appear on sun damaged skin areas, on a precursor lesion: lentigo maligna. Clinically it presents asymmetrical wide spread pigmented macula witch grow slowly in few years. *(Fig. 3)* The main feature of this clinical subtype is prolonged horizontal development of melanoma in situ and so that an important number of cases has a good prognostic after surgical excision.

Acral lentiginous melanoma represents 2-8% of melanomas and this irregular dark macula is located on the palms, soles and nail bed.

Mucosal lentiginous melanoma appears at the oral, genital and anal mucosae like a pigmented asymmetrical flat dark lesion.

Amelanotic melanoma put a serious diagnosis problem due to the clinical aspect: red macula or papula witch has a fast growth.

Clinical tumor examination provide diagnosis in 60% cases.

Imaging diagnosis

Dermoscopy and computed dermoscopy

Dermoscopy and computed dermoscopy show the skin tumor with a 10-20-fold magnification and detected the early stage

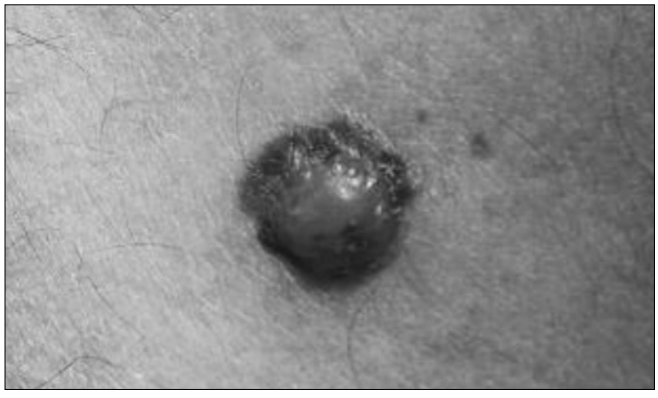


Figure 1. *Clinical aspect of nodular melanoma*

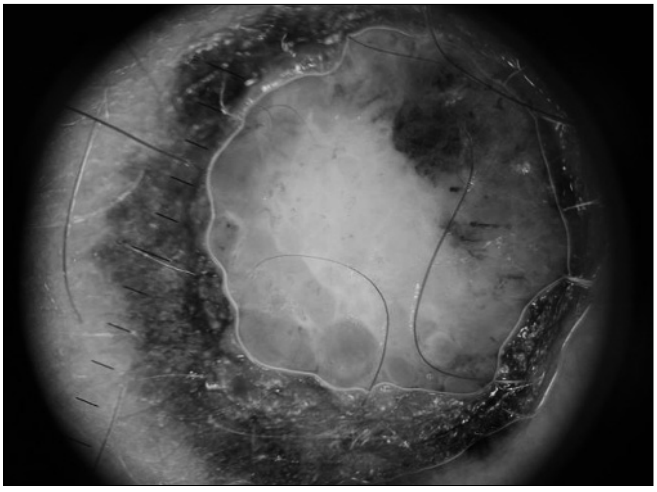


Figure 2. *Dermoscopic aspect of nodular melanoma*



Figure 3. *Clinical aspect of lentigo maligna melanoma*

cutaneous melanoma. *(Fig. 2,4)* During the latest years dermoscopy prove a very useful non-invasive and cheap technique for pigmented skin lesion and due to the pattern analysis can made the differential diagnosis between benign melanocyting tumors and melanoma. (3)

Digital dermoscopy is the latest non invasive imaging method and include an optical system of lens connected with a computer, so the images of pigmented lesions can be stored. The device can take micro- and macroscopic pictures with high resolution. Dermoscopic images of skin lesion can be compared during follow-up examination and small changes are easy detected. This new imaging diagnosis method improves early stage melanoma detection and diagnostic accuracy.

Digital image analysis based on computer diagnostic program and can offer a high sensitivity and sensibility automatic diagnosis of melanoma. (4)

Chest X-ray

Chest X-ray is a common imaging technique, useful for lung witch are visceral site for distant metastases and provide a common preoperative screening investigation.

Computer tomography

Computer tomography (CT) identify visceral and lymph node metastases. Chest CT play an important role in evaluation of pulmonary, pleural, mediastinum and hilar lesions and is very sensitive for small nodes ($\leq$1 cm). Abdominal CT with contrast substance find liver metastases witch are hypervascular and is considered single-imaging technique for the abdomen. (5) CT is a superior investigation method despite relative high cost, high radiation dose and the results are not specific for melanoma.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is non invasive imaging technique most sensitive than CT for the brain, liver and skeleton metastases. Main disadvantages are represented by the limit of scanning the entire body like CT, prolonged period of one organ investigation, artefacts by breathing or cardiac movement and high cost. So that MRI is suitable for one organ investigation.

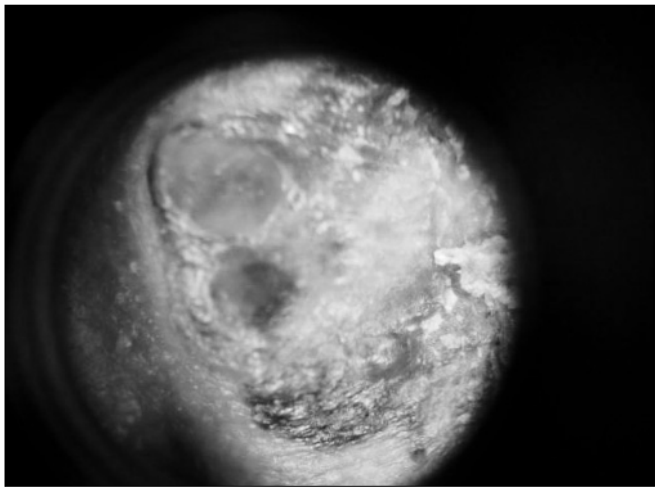


Figure 4. *Dermoscopic aspect of lentigo maligna melanoma*

Ultrasound

Ultrasound is a non-invasive, inexpensive imaging investigation of cutaneous melanoma, regional nodes and abdominal organs. Ultrasound can detect cutaneous lesion of melanoma and studies proved 100% sensitivity for differential diagnosis with basal cell carcinoma, but dermoscopy offers more accurate images. Lymph node basins can be evaluated by this technique witch is more accurate than clinical examination with 99% sensibility and specificity. (6) Abdominal metastases are detected by ultrasound. This procedure is strong depended by the technician and is limited by the local condition: intra-abdominal gases or a fat abdominal wall.

Sentinel lymph node biopsy

Sentinel lymph node biopsy (SLNB), as proposed initially by

Morton et al., is actually the gold standard for melanoma investigation and is included in AJCC classification. The first station of lymph drainage tumor is in sentinel node, so that the probability to be the first metastatic site is maximum. The procedure consist in injection of Technetium 99 radio colloid around the tumor and after 2h the image of sentinel lymph node is taken by a low-energy, high-resolution collimator. After that the localization of SLN the above skin is marked. Biopsy of SLN is followed by histopathological examination. This method is indicated in patients with intermediate-risk: lesion thickness of 1-4 mm and high-risk lesion >4 mm.

Pre-operative lymphoscintigraphy is an important step for detection sentinel lymph node biopsy and increased the accuracy of the investigation at 95-99%. (7) This technique is the most accurate method for micrometastases ≤ 4 mm, despite the adverse effects, like radiation exposure or potential local complication(lymphoedema).

Vital blue dye is injected peritumoral for intraoperative visualization of sentinel lymph node and is easy, inexpensive and helpful procedure.

SLNB became a standard in melanoma staging and is important for treatment decision, mostly adjuvant schedules. A positive results is followed by elective lymph node dissection.

Positron-emission tomography

Positron-emission tomography (PET) is the last imaging method used in staging of melanoma. It is based by the affinity of tumor cells for the radio-labeled glucose analogue (18 –FDG) so that the capacity of metastases detection is superior than CT. PET can detect tumor lesion >4 mm and PET sensitivity increase with clinical stage: 0% in stage I, 24% in stage II, 81% in stage III and 100% in stage IV (Wagner et al). PET can not identify brain metastases due to the normal high tissue activity witch is comparable with tumor tissue. Recent research compared PET and SLNB sensitivity: just 12% of SLN were detected by PET and this technique is relevant when melanoma disseminates at SLN and suspect visceral metastases. Also PET begin to detect intraoperative tumor cells.

Positrone-emission tomography and computed tomography

Positrone-emission tomography and computed tomography (PET/CT) combine simultaneously the two imaging technique: the PET images are mapped in CT images so the tumor location is more specific. *(Fig. 5,6,7,8)* Despite expensive cost PET/CT is used for diagnosis of high-risk melanoma (stages II-III) because it was proved high sensitivity (99%) compared with PET only. This recent imaging technique proved usefulness for detection the distant metastases and after that the decision for surgical excision. The main disadvantage is the misinterpreted the very small structure as metastases and so that is required to compare the results with previous images.

Serological biomarkers

Tumor cells and related cells have capability to produce

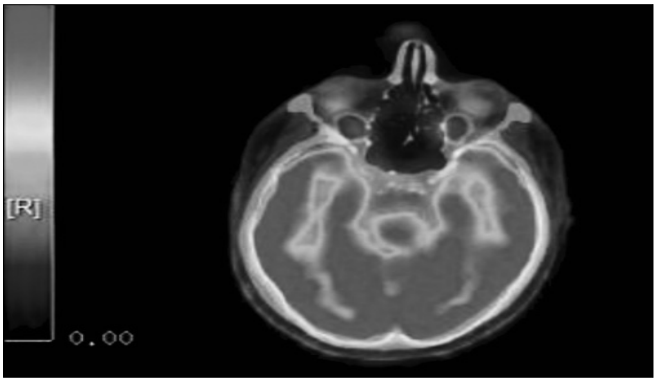


Figure 5. *PET/CT follow up melanoma, brain level*

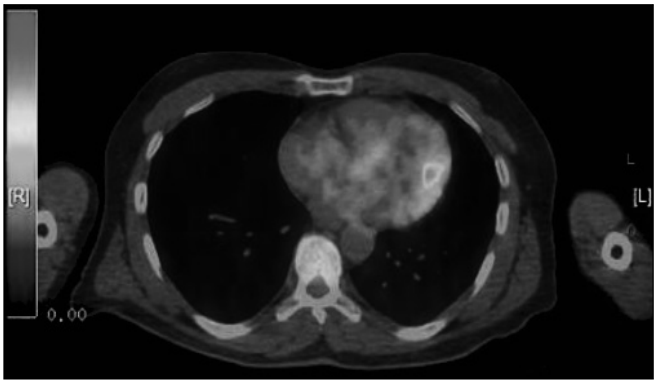


Figure 6. *PET/CT follow up melanoma, thoracic level*

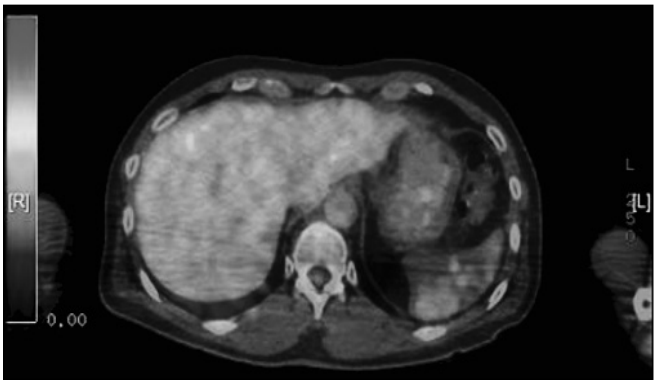


Figure 7. *PET/CT follow up melanoma, abdominal level*

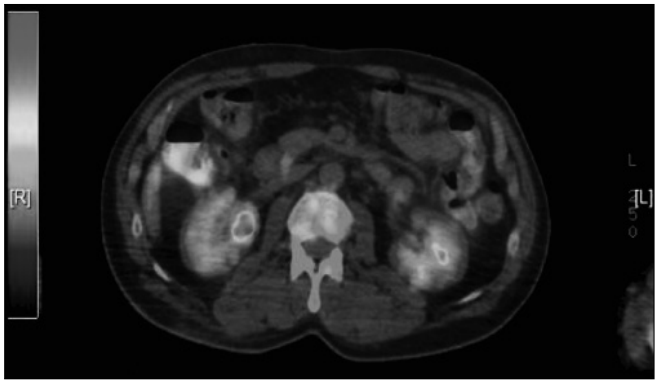


Figure 8. *PET/CT follow up melanoma, abdominal leve*

specific proteins witch can be determined in blood and the levels of biomarkers are very useful in melanoma diagnosis and prognostic of the disease.

Several circulating molecules are monitoring in melanoma: LDH, S100, C-reactive protein, MIA (melanoma inhibitory activity protein), TA90 (tumor-associated antigen 90 imune complexes), IL6. In early stages of melanoma this serological parameters have no significance, in fact principal disadvantage and limit of this investigation.

LDH is an unspecific biomarker in different types of tumor, but inexpensive and easy detected parameter in laboratories. High levels in the latest melanoma stages suggests tumor progression and negative survival prognostic. Many studies demonstrated that LDH is the main predictive factors in stage IV melanoma and also a play an important role in therapy response.

C-reactive protein

C-reactive protein has a high level in stage IV melanoma and represent a negative predicted factor, correlated with short survival rate and with progression of metastasis during therapy. Prospective studies demonstrated usefulness of CRP and LDH in melanoma staging and superiority of the first in differentiation of stage I-III and IV.

S100

S100 is a serum protein which was first identify in cultured melanoma cells and after that was isolated in peripheral blood. The level of this tumor marker is in direct relation with clinical stage, survival rate, presence of the metastases and melanoma relapse .Clinical trials demonstrate the importance of this biomarker in melanoma management and nowadays is frequent determined in specialized laboratories. S100 lower baseline level is correlated with prolonged survival. (8)

Melanoma inhibitory activity protein

Melanoma inhibitory activity protein (MIA) is produced by the melanoma cells and represent a new important biomarker. Elevated serum levels are in direct relation with tumor progression, the greater value being in IV stage. During follow-up high level of MIA suggests presence of the metastasis. (8)

IL6

IL6 is an immunological parameter with increased serum levels in IV stage melanoma and play an important role in evaluation of tumor progression and survival rate.

ProWling serum proteomic profiling

ProWling serum proteomic profiling is the newest methodology witch provide screening of entire serum proteome, in fact different biomarkers mentioned above.

Using proteomic profiling, Findelstein et al. reported an new biomarker, serum amyloid A and correlated high level with poor survival rate. (8)

A combination of serum biomarkers correlated with AJCC staging, play an important prognostic role, very useful in latest melanoma stages, monitoring after surgical excision of primary tumor in I-III stages and evaluation therapy in advanced stages. But there are two principal limits: none a single marker can be considered specific for melanoma and predictive significance are just for latest stages: presence of metastases or good therapy response.

TNM staging according to the AJCC, 2001

This new staging system contains as principals parameters Breslow's criteria, Clark's level and new element, ulceration, witch is determined by histopathology. *Table 1*

Table 1.

TNM staging of melanoma, AJCC,2001			
Stage	**Primary tumor (pT)**	**Regional lymph node metastases (N)**	**Distant metastases (M)**
0	In situ tumor	None	None
IA	≤1 mm, without ulceration	None	None
IB	≤1 mm with ulceration or Clark level IV or V 1,01-2 mm, no ulceration	None	None
IIA	1,01-2 mm with ulceration	None	Non
	2,01-4 mm, no ulceration	None	None
IIB	2,01-4 mm with ulceration	None	None
	>4 mm, no ulceration	None	None
IIC	>4 mm, with ulceration	None	None
IIIA	Any tumor thickness, no ulceration	Micrometastases	None
IIIB	Any tumor thickness with ulceration	Micrometastases	None
	Any tumor thickness, no ulceration	1-3 macrometastases	None
	Any tumor thickness with or no ulceration	None but satellite and or in transit metastases	None
IIIC	Any tumor thickness with ulceration	1-3 macrometastases	None
	Any tumor thickness with or no ulceration	>4 macrometastases, or satellite and or in transit metastases, or lymph node involvement beyond capsule	None
IV			Distant metastases

Prognostic factors

According to AJCC classification, rate of survival at 5 years is depending of the stage: for stage 0 is 97%, for stage I is 92,5% and decrease at 75% at stage II, 49% at stage III and 18% at stage IV.

In additionally of the system there are some negative prognostic factors:

- Age >60 years;
- Sex: male gender;
- Race: non-caucasian;
- Location: scalp,middline of the trunk,the hands and the feet;
- Histological aspects: nodular or acral subtypes, presence of ulceration, vertical growth phase, excessive mitotic activity, presence of cell nests (microscopic satellites) and vascular penetration;
- Presence of multiple visceral metastases.

Surgical management

Surgical excision of primary cutaneous melanoma is certain method of treatment, always under general anesthesia. The resulting defect will be cover using one of main method: direct suture, free skin graft, local or distant flap.

The limits of primary tumor excision

How wide and deep must to perform an adequate surgical excision? This question has a lot of answer during the last century: begin with W. Handley in 1907 who recommended safety margins of 2,54 cm, in 80s the attitude was more radically: excision limits of 5 cm.

TheWHO Melanoma Group performed large randomized study witch proved that the disease free period was similar for 1cm margins of excision primary melanoma of 2 mm thickness likewise 3 cm borders. Also some recent studies recommended safety margins: 0,5 cm for in situ tumor, 1 cm for ≤ 2 mm tumor thickness and 2 cm for >2 mm tumor thickness. If 1cm limits of excision are safety remain an unsolved problem because the presence of cell nests closely to the primary tumor. The excision depth level is subfascial.

Concerning with this unsolved question, we are now involved in project with technological researchers to study using spectrophotometry and microphluid biochip to detect the proper tumor border, including presence of peritumoral neoplasic cells.

Mohs micrographic surgery(MMS)

MMS proved that is a very helpful method in non-melanoma skin malignancies grace to the entire margins of tumor are examined. The aim of MMS is to evaluate the entire tumor border and the deep. Excised lesion is histopathological analyzed. If remain tumor tissue the procedure is repeated. This treatment of melanoma technique is more laborious, but recent studies has shown that use of imunostains (especially MART-1) of frozen section improve the results. MMS play an important role in melanoma management particularly ill-define lesion. (9)

Adjuvant melanoma treatment

In stage II and III disease adjuvant schedules are chemotherapy, unspecific immunotherapy and interferon.

Dacarbazine is the most used chemotherapeutic agent, but with poor response rate: only 15-20% for 5-6 month and just 1-2% patients achieved a long term response. Other chemotherapeutic agents like fotemustine, vindesine or temolozomide have no superior respose then dacarbazine. Current poor results require new protocols of multiagent chemotherapy.

Unspecific immunotherapy is represented by the BCG, but no clinical trials proved the real benefit.

Interferon-α2b improve the immune response against the tumor cells and is used as an important adjuvant in stage II and III melanoma treatment. Today are applied different dosage schedules: high-dose of IFN α2b in US and in Europe low-dose of IFN α2b. The Eastern Cooperative Oncology Group Clinical trials has demonstrated prolonged free-disease survival in high-dose of IFN α2b schedules. Comparative data regarding both adjuvant therapeutic protocols are included in 11 clinical trials including 4878 show no relevant differences of results, but adverse effects are more severe with high-dose schedules. So far only IFN α2b brings a benefit in life prolongation for the patients with cutaneous melanoma.

Evan combined treatment protocols between chemotherapeutic agents and IFN α2b does not increased the survival rate, in addition present higher toxicity.

Advanced therapeutic procedure

Nanotechnology

Nanotechnology is the most recent science of nanoparticles with application in melanoma treatment. The research is now on mice with melanoma: hollow gold nanospheres equipped with a targeting peptide find and penetrate melanoma cells and after that photothermal ablation destroyed tumor. The major disadvantage of nanoparticles even if gold nanospheres are very small (40-50 nm) is the presence of biological filters like spleen and liver where untargeted particles are destroyed. The targeted nanospheres gathered in the tumor and after that at spleen and liver. Only tumor cells penetrated by the targeted nanospheres and after that treated with near-infrared light were killed.

Gene therapy

In 2007 Rosenberg et al. propose an advanced therapeutic method witch transform normal lymphocytes in tumor fight cells: after the sampling, the lymphocytes are infected in vitro with a retrovirus containing genes for specific proteins, called T cell receptors (TCRs). These receptor proteins are expressed on the external surface of the lymphocytes and have the property of binding molecules on the surface of the tumor cells and after that the lymphocytes kill the tumor cells. 17 patients with advanced metastatic melanoma treated by this method presented tumor regression and remain disease-free for one year. (10)

No adverse effects were reported. Improvement of method using retroviruses is research.

Follow up protocols

Prospective study performed by German researchers using 2000 patients proved the importance of a rigorous follow up plan. (11, 12) Physical examination are performed at every 3 month during first 5 years and every 6 month during 6-10 years and include: examination of surgical scar, entire skin examination for new or recurrence melanoma and palpation of lymph nodes, liver and spleen.

Blood test including LDH, alkaline phosphatase, protein S100 and liver function test are performed simultaneous with physical examination. Chest X-ray and abdominal ultrasound are recommended only for patient with stage III melanoma twice a year during 10 years.MRI can identify abdominal and soft tissue metastases >1 cm. PET-CT technique provide a high resolution and sensitivity for the recurrences of melanoma and occult metastases due to the detection the higher metabolic rate and glucose metabolism in the tumor cells.

This advanced imaging techniques are very useful during follow-up schedules, but in the mean time can offer a lot of false - positive results due to the very small structures. So that a rigorous examination of previous imaging results can avoid mistakes.

Conclusions

Despite the recent new knowledge and technological equipments melanoma still remain an incurable disease in advanced stages with lymph node dissemination or visceral metastases. The aim of current treatment is only the prolongation of survival.

A real progress is for early diagnosis of lesion <1 mm thickness using dermoscopy and digital dermoscopy when the cutaneous melanoma is cured. Dermoscopy as screening method for congenital nevi can be considered the unique way to prevent melanoma development.

Sentinel lymph node biopsy has demonstrated to be a trusted diagnosis procedure for >1 mm thick melanoma without clinical sign of regional adenopathy, so in many cases N_0 became N_1.

A combination of serological biomarkers play an important role in stage I-III melanoma monitoring after excision and prediction of metastases growth.

Current staging is based on AJCC 2001 classification and besides tumor thickness additionally contains histopathologic ulceration in stage I and II and lymph node micro- and macrometastases in stage III.

Recent imaging technique like PET or PET/CT together with MRI and CT can diagnosis very early metastatic lesion and are very important in management of melanoma and the decision of surgical removal.

Surgical removal with 1-2 cm margins of early stage tumor represents the single method of treatment. Mohs micrographic surgery for lesion with undefined borders improve the results, but the technique of frozen immunostains (especially MART-1) are available in specialized centers.

Present treatment of advanced melanoma doesn't ensure healing offers, just short term prolongation of life. Adjuvant therapy offers poor results in free disease periods and life span.

Melanoma treatment remains an unsolved problem yet.

New therapeutic methods are now researched, including monoclonal antibody, gene therapy or golden nanosphere and represented maybe the future in melanoma treatment.

References

1. Bosetti C, La Vecchia C, Naldi L, Lucchini F, Negri E, Levi F. Mortality from cutaneous malignant melanoma in Europe. Has the epidemic leveled off? Melanoma Res. 2004;14(4):301-9.
2 Weinzweig J. Malignant melanoma. Plastic surgery secrets. 1999;8:38-45.
3. Zalaudek I, Ferrara G, Argenziano G, Ruocco V, Soyer HP. Diagnosis and treatment of cutaneous melanoma: a practical guide. Skinmed. 2003;2(1): 20-31; quiz 32-3.
4. Glud M, Gniadecki R, Drzewiecki KT. Spectrophotometric intracutaneous analysis versus dermoscopy for the diagnosis of pigmented skin lesions: prospective, double-blind study in a secondary referenc center. Melanoma Res. 2009;19(3):176-9.
5. Garbe C, Eigentler TK. Diagnosis and treatment of cutaneous melanoma: state of art 2006. Melanoma Res. 2007;17(2):117-27.
6. Dancey AL, Mahon BS, Rayatt SS. A review of diagnostic imaging in melanoma. J Plast Reconstr Aesthet Surg. 2008;61(11): 1275-83. Epub 2008 Aug 10.
7. Chakera AH, Drzewiecki KT, Eigtved A, Juhl BR. Sentinel node biopsy for melanoma: a study of 241 patients. Melanoma Res. 2004;14(6):521-6.
8. Mouawad R, Spano JP, Khayat D. Old and new serological biomarkers in melanoma: where we are in 2009. Melanoma Res. 2010;20(2):67-76.
9. Whalen J, Leone D. Mohs microgeaphic surgery for the treatment of malignant melanoma. Clin Dermatol. 2009;27(6):597-602.
10. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients mediated by transfer of genetically engineered lymphocytes. Science Express. Online August 31, 2006.
11. Francken AB, Hoekstra HJ. Follow-up of melanoma patients: the need for evidence-based protocols. Ann Surg Oncol. 2009;16(4): 804-5. Epub 2009 Feb 3. Comment on: Ann Surg Oncol. 2009 Mar;16(3):571-7. Ann Surg Oncol. 2009 Apr;16(4):941-7.
12. Constantinidou A, Hofman M, O'Doherty M, Acland KM, Healy C, Harries M. Routine positron emission tomography and positron emission tomography/ computed tomography in melanoma staging with positive sentinel node biopsy is of limited benefit. Melanoma Res. 2008;18(1):56-60.