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Wide instrumental screening in monitoring early melanoma

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Summary. *Aim:* Instrumental skin screening is helpful for an early diagnosis of melanoma. The authors have drawn up a diagnostic protocol based on clinical history and simultaneous dermatoscopy, thermography and ultrasound assisted objective examination. *Patients and methods:* 337 patients were recruited at the Second Opinion Medical Office (Modena, Italy) to perform nevi mapping. 71 patients with potentially dangerous nevi (according to the American Cancer Society – ACS) were selected for follow up. The cancer family history, phototype, density and oncological risk were reported. A clinical evaluation of morphological skin nevi was performed by dermatoscopy, dynamic thermography (cooling-down 5°C for 20 seconds) and the ultrasound method. Moreover patients were advised immediately to send photos of morphological mutations or symptoms connected with their nevi in the following months. *Results:* A total of 99 significant skin nevi were identified. Of these 60 were hotter than controls, and 39 had the same temperature. Cooling-down produced a mean temperature reduction of 4.5°C and a recovery of 2°C after 30 seconds. *Conclusions:* Conclusions of the present work suggest an innovative screening protocol applicable on a healthy young population, sensitive to the theme of precocious cancer diagnosis.

Key words: melanoma, screening, dermatoscopy, thermography, follow-up

«Screening strumentale ad ampio spettro per sorveglianza e diagnosi precoce del melanoma»

Riassunto. *Obiettivo:* Lo screening strumentale cutaneo della pelle è importante per la formulazione di una diagnosi precoce di melanoma. Gli autori hanno voluto elaborare un protocollo diagnostico basato sulla utilizzazione sinergica della dermatoscopia, della termografia dinamica e della ultrasonografia nella valutazione clinica delle lesione pigmentate della pelle. *Pazienti e metodi:* 337 pazienti si sono rivolti al Poliambulatorio del Secondo Parere (Modena, Italia) per effettuare una mappatura dei nei. Di questi, 71 sono stati selezionati per una indagine più approfondita. Sono stati riportati la storia oncologica familiare, il fototipo (F), la densità dei nevi (D) ed il rischio oncologico (N). Una indagine dermatoscopica, termografica dinamica con raffreddamento (5°C per 20 secondi) ed ultrasonografica è stata effettuata. I pazienti, inoltre, sono stati istruiti ad inviare documentazione fotografica, previa modificazione morfologica delle lesioni, nei mesi successivi. *Risultati:* Un totale di 99 lesioni cutanee degne di approfondimento diagnostico sono state identificate; di queste 60 si sono dimostrate ipertermiche all'analisi termografica e 39 invece sono risultate essere normotermiche. Il raffreddamento ha determinato una riduzione media della temperatura di 4,5°C ed un recupero di 2°C in 30 secondi. Al follow up hanno aderito 13 pazienti. Di questi, 3 pazienti si sono sottoposti a rimozione di nevo segnalato, con diagnosi di 1 melanoma, 2 pazienti hanno effettuato una seconda visita di controllo, mentre i restanti 5 pazienti hanno provveduto ad inviare documentazione fotografica. *Conclusioni:* I risultati dello studio dimostrano l'applicabilità di tale protocollo innovativo ad una popolazione giovane, sempre più sensibile al concetto della telemedicina.

Parole chiave: melanoma, screening, dermatoscopia, termografia, follow-up

Introduction

Every year about 68,000 malignant melanomas are diagnosed. The incidence of death is 12.74% and is related to geographic location (1). The incidence rate is higher in Norway and Sweden and less so in Italy and Poland. The mortality rate is quite constant throughout the world, but the incidence rate increases in the male gender.

Gene mutations are the most important etiological factors behind malignant melanoma. In most cases heterozygote CDKN2A mutation is involved in familial melanoma (2). Other genes involved in malignant transformation of cells are cadherins and integrins (3), C-kit (4) and BRAF (5) (Figure 1). CDKN2A mutations interfere in the transition from G1 to S phase by Retinoblastoma (RB), p16 and cyclin D1 inhibition, as well as promoting cellular replication (6). BRAF is a kinase protein involved in the BRAF - MEK - ERK pathway. The protein activate Microphthalmia-associated Transcription Factor (MITF) involved in proliferation and differentiation of melanocyte cells (7) and probably in malignant cell transformation BRAF-stimulated MITF degradation (8). C-kit is the receptor of stem cell factor (SCF). It regulates migration and survival of melanocyte cells in the MAPK pathway (9). Mutations of MC1R are not able to stimulate melanin production and consequently the skin is not adequately protected from UV (10). Both MC1R mutations and overexposure to the sun are responsible for malignant BRAF activation. The PTEN – PI3K – AKT- mTOR pathway is also involved in cellular proliferation by MITF regulation. AKT activation promotes replication and inhibits apoptosis by the inactivation of Bad and Bax (11).

Non-invasive diagnostic criteria

Dermatoscopy

Visual analysis of skin lesions aided by the highest optical resolution of dermatoscopes is considered the classic diagnostic method for melanoma.

The most modern dermatoscopes are based on a polarized-light manual microscope connected to a computer. Dermatoscopes allow a 5 to 30% improvement in the perception of skin lesions, as compared to human observation (12) and 80% sensitivity in melanoma diagnosis (13). The morphological parameters utilized in diagnosis are summarized in Figure 2 (ABCDE criteria).

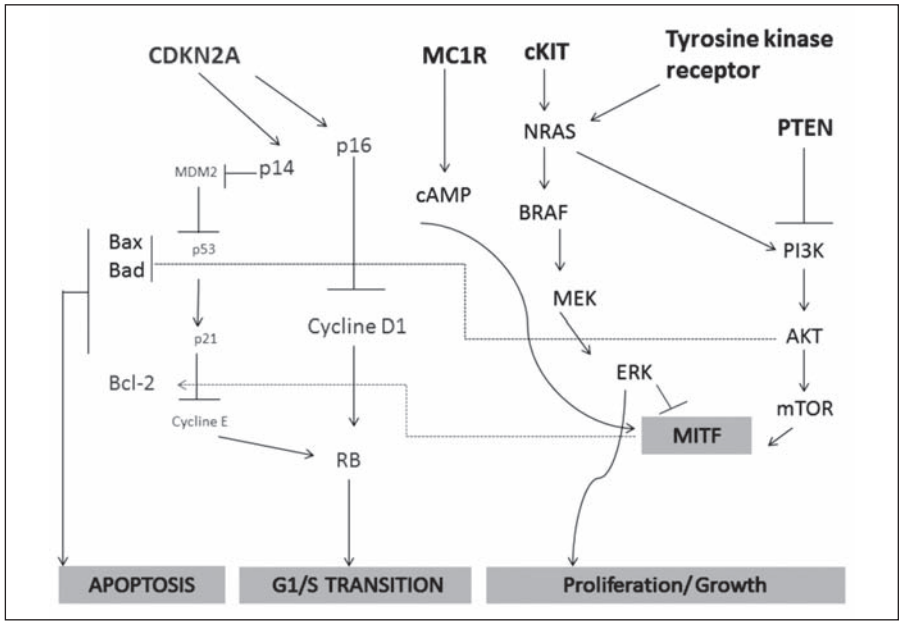

Figure 1. Molecular pathways involved in the etiology of melanoma.

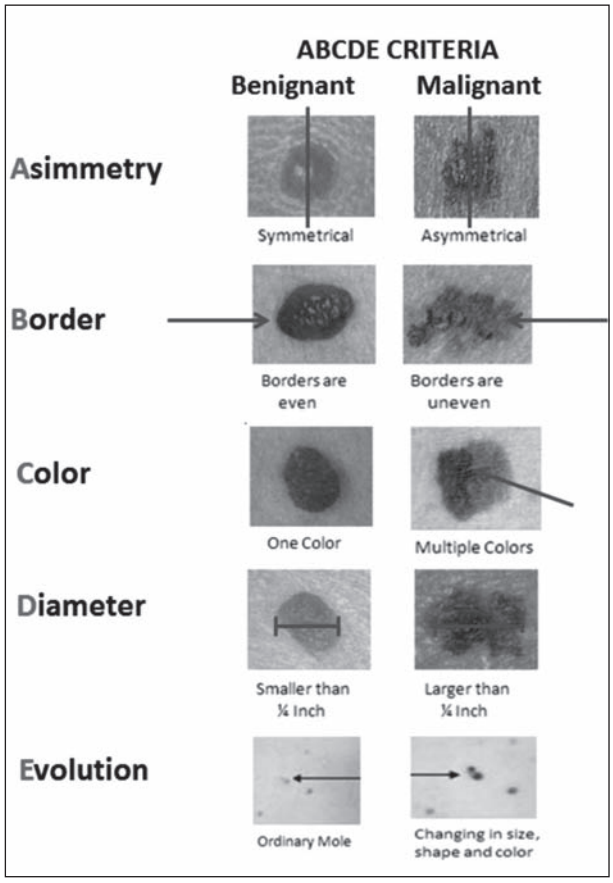

Figure 2. ABCDE criteria in the screening of skin lesions. Benign (left) and malignant lesions (right) are compared.

Diagnostic thermometry and thermography

Malignant cells in melanoma consume high levels of glucose because the metabolism and the angiogenesis process increase. Consequently the temperature of malignant skin lesions is higher than benign skin lesions and surrounding skin (14, 15). Thanks to laser instrumentation (laser thermography) it is possible to reveal the increase in temperature and to extend this method to clinical monitoring of other systemic diseases, e.g. sclerosis, diabetes, and hypertension. Dynamic thermography, thanks to cold stimulation, allows to discriminate malignant from benign lesions.

Ultrasound methods

High-resolution ultrasound echography enables to explore the morphology, the thickness and the extent of skin lesions. The ultrasound imaging method is based on wave reflection through tissues and conversion of them into images. High-frequency ultrasounds analyze the epidermis (100 MHz), while low-frequency ultrasounds penetrate into the derma and deeper organs (20-25 MHz). Clinically, high-frequency ultrasounds are employed in melanoma diagnosis, squamous cell carcinoma (16), margin dimensions of lesions (17) and skin thickness (18).

Ultrasound methods, thanks to contrast agents, assess blood flow and volume near the malignant area. Forsberg *et al.* (2002) in a murine model in which human melanoma cells were implanted, considered ultrasound investigation as an angiogenesis marker comparable to cyclooxygenase -2 (COX-2) expression (a malignant angiogenesis marker) (19).

Ultrasound methods should be employed to reveal metastasis in sentinel lymph nodes both in melanoma and in breast cancer. CELUS is an ultrasound probe (Contrast enhanced lympho-ultrasonography) which allows one to visualize lymph metastasis in nodes thanks to contrast agents such as Sonazoid (GE Healthcare, Oslo, Norvey), Luminity (Bristol-Myers Squibb Medical Imaging, Billerica, MA) and SonoVue (Bracco, Milan, Italy). They consist in lipid-coated particles of 2-2.5 μm diameter and high-reflectance resolution (20). The probe discovered 22/26 malignant sentinel lymph nodes in pigs (21), while in humans the results were not significant. CELUS application has not been extended to the clinical field probably because its contrast agents were not specific to human administration (20, 22).

DOPPLER ultrasounds reveal cell movements in tissues and blood flows, emitting a specific color-code to show the direction of and volume blood flow (23).

Materials and methods

Subjects

All the subjects (N= 337) accepted informed consent before investigations started. The subjects were patients from the Second Opinion Medical Office in Modena (Italy) which performed a nevi map from June to November 2013. 71 subjects were selected because they manifested significant skin lesions.

Methods

Medical history questionnaire

A questionnaire was distributed to all patients. It collected personal data, present and/or past oncologic familial history, maximum familial age. During the medical examination we characterized the photo-type (F), the density of skin lesions (D) and the oncological risk (N) according to nevi characteristics and the anamnesis performed. The oncologic risk was assessed as follows: 1= low risk; 2= moderate risk; 3= medium risk; 4= high risk; 5= very high risk. The oncologic risk was assessed according to the patient photo-type, the density of the nevi and anamnesis of previous personal oncologic history. Consequently, patients with low photo-type 1, a very high density nevi and a previous oncologic personal history had score 5 of oncologic risk. Patients with photo-type 1, medium nevi density and no previous oncologic personal history scored oncologic risk 4. Score 3 of oncologic risk was attributed to patients with photo-type 2 and medium/high density nevi, in the absence or presence of a previous oncologic personal history. Score 2 was attributed to patients with photo-type 2 and low density nevi, with or without a previous oncologic personal history. Finally, score 1 was given to patients with photo-type 3. We configured the risk factors highlighted in the scientific literature in a diagnostic algorithm: they included the photo-type, the density, and the oncologic personal history (24, 25).

Macroscopic photography

Different photographs of skin lesions were collected by a Canon DS 126191 digital camera (Canon Italia S.p.a., Cernusco Sul Naviglio, Milan, Italy).

Dermoscopy

An Optilia dermatoscopic instrument (Optilia Medical, Sollentuna, Sweden) was employed to analyze skin lesions. A different number of dermatoscopic images were collected, according to the density (Fig. 3).

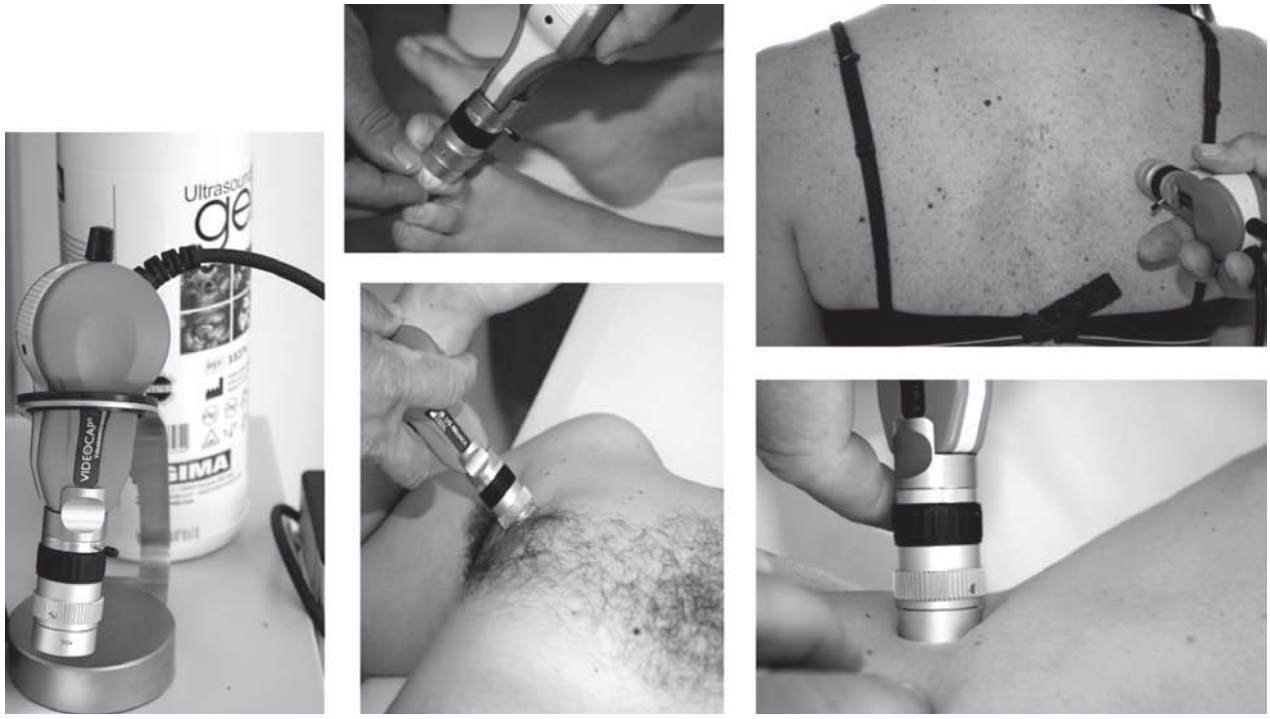

Figure 3. Optilia dermatoscopy, Optilia Medical, Sollentuna, Sweden and dermatoscopic procedure.

Dynamic thermography

Only for morphologically atypical lesions thermography analysis was conducted (Flir i40, Flir Systems Srl, Limbiate (MI); Fig. 4). The instrument is able to detect temperatures between -20°C and + 350°C; it possess an integrated digital camera (resolution IR 120x120 pixels), double-LED light, and FLIR Fusion Picture-in-Picture (FPiP) function, which permits one to observe IR images overlying visible images. The precision is +2%, and the thermal sensitivity up to 0.1°C. Secondly, we evaluated the temperature of a symmetrical skin area (control measure). The thermograph was switched on at least 15 minutes before starting medical examination, allowing for adaptation of the instrument to the room temperature (20°C). The individualized skin lesions were cooled down by a metal plate (20 seconds, 5°C) and lastly the temperature was recorded to evaluate the 30 second recovery.

Ultrasound echography

A dermal echography was performed by Dermalab® Combo scan (Cortex Technology ApS, Hadsund, Denmark; Fig. 5) to evaluate skin thickness (data not analyzed for lack of a significant sample).

Figure 4. Thermal Imaging Camera Flir i40, FLIR Systems Srl, Limbiate (MI), thermograph analysis and cooling-down.

Figure 5. Dermalab Combo, Cortex Technology Aps; Hadsund, Denmark and procedure.

Statistical analysis

Microsoft Excel 2010 (Office package) software was employed to perform statistical analysis. Gender, age, photo-type and oncological risk assessment were evaluated. Also mean temperature of skin and control lesions, % temperature variation of skin lesions compared to control lesions, temperature recovery after cooling-down, body area analysis of significant skin lesions, familial oncologic history and maximum familial age were also considered.

Results

Characteristics of the sample

71 patients (males=36 and females=35; 50.7% and 49.3% respectively) aged between 13 and 63 years old (medium age 38 years old) were selected from 337 patients. Photo-type results (F:1-6) are shown in Figure 6. No patients with photo-type 4, 5 and 6 were distinguished. Results related to oncologic risk assessment (N:0-5) are summarized in Figure 7. No patients with N=0 were identified.

Skin lesion results

99 skin lesions were identified in 71 selected patients. Body areas, in which lesions were distinguished, are summarized as a chart in Figure 8.

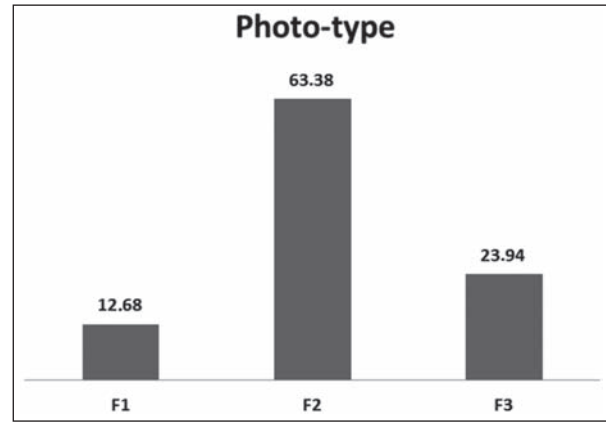

Figure 6. Incidence rates of photo-type in selected patients (N= 71).

60.87% (60 lesions) were hotter than controls measured by thermography. The variance in mean temperature was 0.67°C (0.02%). 39.13% (39 lesions) had the same temperature as control lesions. Mean, minimum and maximum values of temperature are shown in Table 1 both for skin lesions and control lesions, while Figure 9 shows mean temperatures.

Figure 10 shows the incidence rates of body areas in which skin lesions were identified, while Figure 11 indicates body areas with skin lesions having the same temperature as control lesions. Cooling down of 60 hotter lesions (5°C for 20 seconds) produced a mean decrease in temperature of 4.5°C and a recovery of 2°C in 30 seconds.

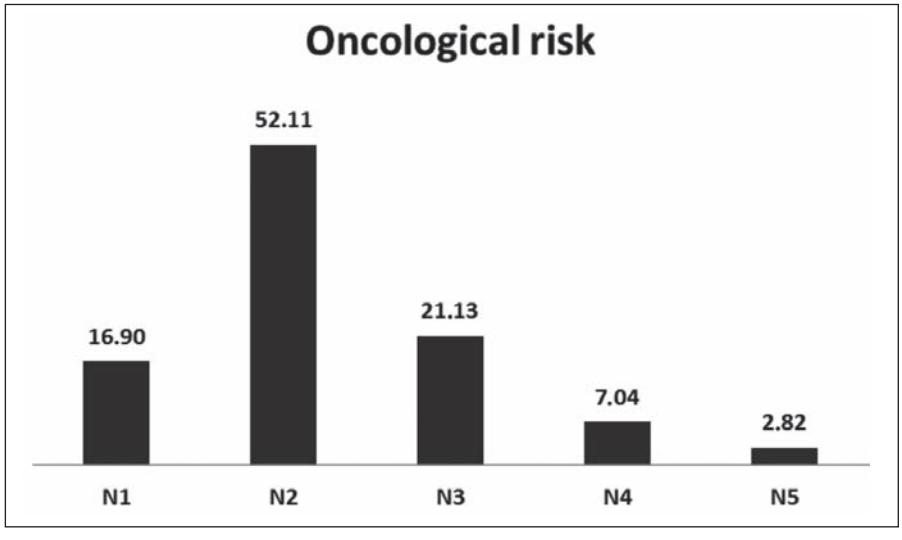

Figure 7. Incidence rates of oncologic risk in selected patients (N= 71).

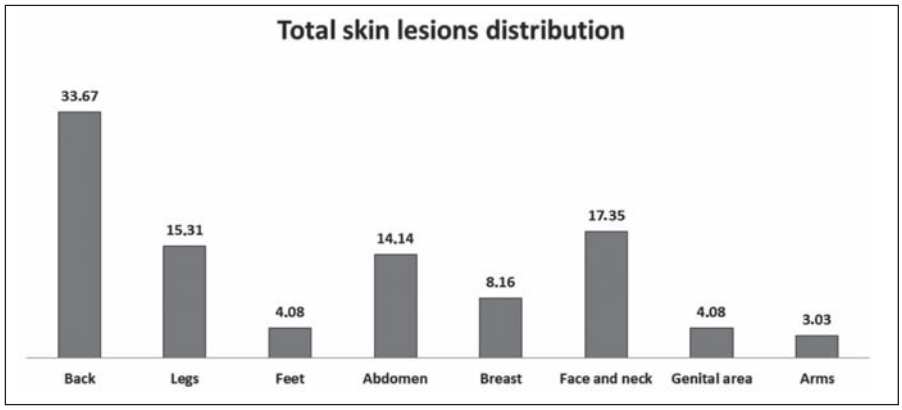

Figure 8. Incidence rates of body areas in which lesions of selected patients were identified (N= 99).

Table 1. Mean, minimum and maximum temperature of skin lesions and control lesions (°C) in selected patients (N= 99).

Type	T medium (°C)	T min (°C)	T max (°C)
Lesion	33.27	29.4	35.9
Control	32.59	27	35.1

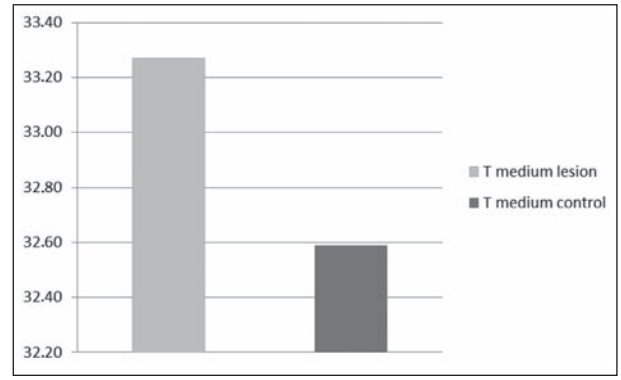

Figure 9. Mean temperature of skin lesions and control regions (° C) in selected patients (N= 99).

Oncologic familial history

255 cases of cancer were recorded in the present and/or past oncologic familial history of 337 patients. Incidence rates are shown in Figure 12. Three patients reported having suffered respectively from thyroid cancer, non-Hodgkin's lymphoma and Hodgkin's lymphoma, from which they recovered in all cases.

Maximum familial age

The maximum familial age was also reported, taking 90 years old as a borderline value. Results relating to 71 selected patients are shown in Figure 13, while Figure 14 exhibits results relating to 337 patients. In both groups, the mean age of patients older than 90 years is 94 years old.

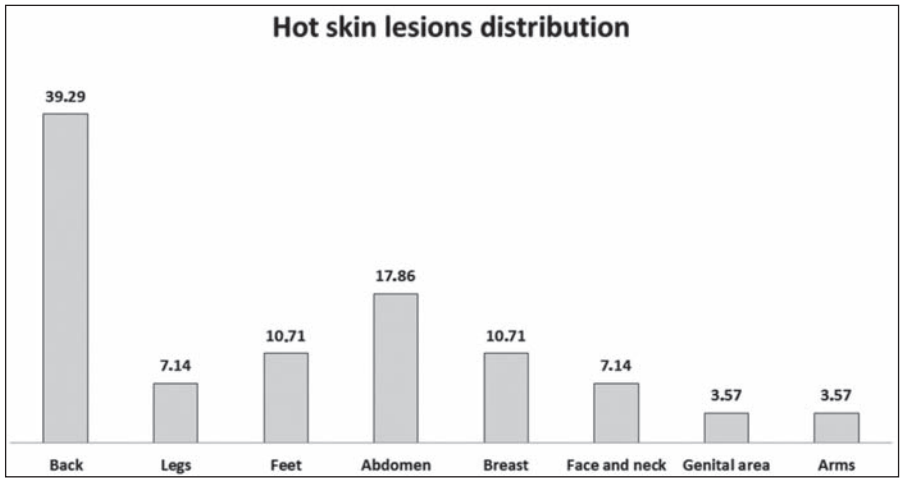

Figure 10. Incidence rates of hotter skin lesions in selected patients (N= 60).

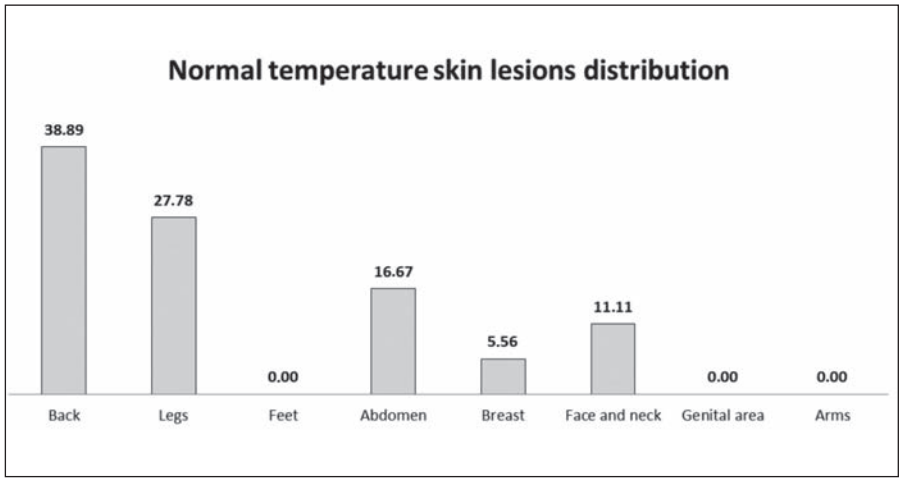

Figure 11. Incidence rates of skin lesions having the same temperature as control lesions in selected patients (N= 39).

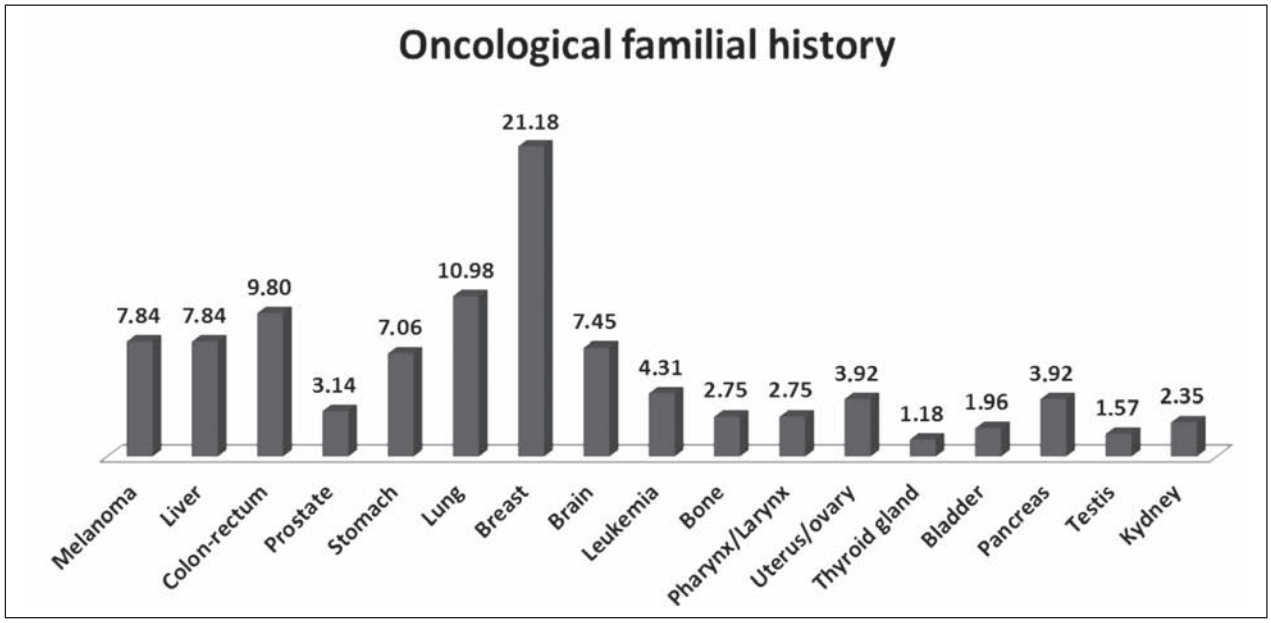

Figure 12. Present and/or past oncologic familial history in 337 patients (N= 255).

Discussion

The study offers interesting points for reflection relating to the risk of malignant degeneration and epidemiological surveillance in healthy young subjects, sensitive to the message of precocious diagnosis. Certainly the most significant instrument of the experimental procedure is the thermograph Flir i40 (Flir Systems Srl, Limbiate, MI). The thermography principle is based on capture of infrared-body energy, transformation into a video signal by a laser source, and a thermal scale equivalent to a color scale. Thermography allows one to visualize the thermal pattern of an extended surface. However, calculations depend not only on the temperature of the surface studied, but also the emissivity, which is the part of energy released from the object compared to the energy released from a black body (an ideal object that adsorbes all electromagnetic incident radiation without reflecting or diffusing energy) at the same temperature. One possible bias of the instrument may be the interference with radiation emitted from the surround-

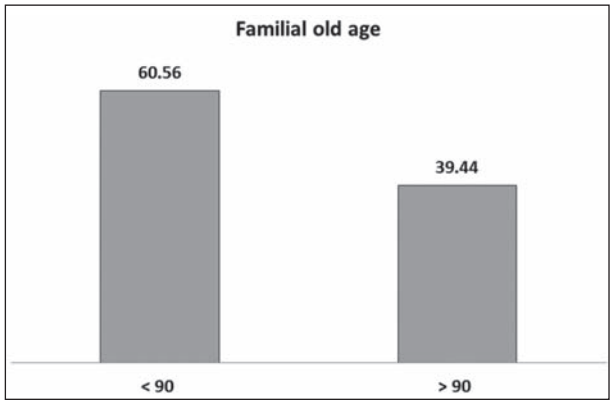

Figure 13. Maximum familial age (N= 71).

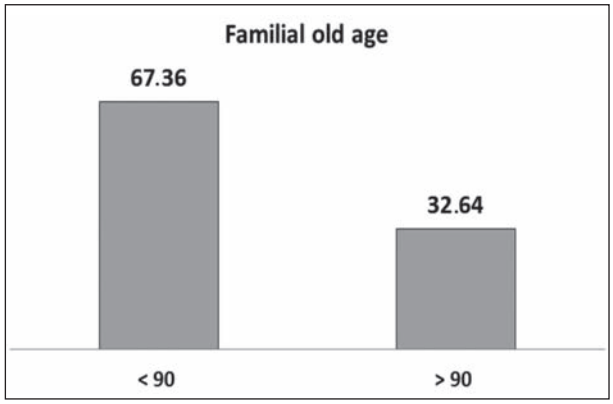

Figure 14. Maximum familial age (N= 337).

ing environment. In the last 20 years this barrier has been resolved with thermographs able to process not only the spatial thermal setting, but also the temporal pattern of the skin. Thermographs possess a camera fitted with a silicon or germanium lens and able to reveal infrared - wave lengths, as well as a laser source and a video instrument. Radiation is decomposed into points or caloric–quantic impulses and these are transmitted to thermal-fanlike elements located in a Dewar vase. This vase contains liquid nitrogen (-196 °C) and permits one to neutralize interference by the surrounding temperature and to achieve maximum sensitivity (26).

Among the most interesting studies, Shada et al. (14) employed an Amber Radiance 1-T IR thermograph (Raytheon, Las Vegas, NV) to reveal melanoma metastasis. It has a specificity of 100% for lesions between 0-5 mm, 98% (> 5-15 mm), 100% (>15-30 mm) and 89% (>30 mm). The sensitivity is 39% (0-5 mm), 58% (>15-30 mm), 95% (>15-30 mm) and 78% (> 30 mm). The prognostic positive value is included between 88% and 100%, while the prognostic negative value is 95% for skin lesions > 15-30 mm and 80% for skin lesions > 30 mm (14).

Whatever the sensitivity of modern thermographs (0.01°C), they are not able to reveal very small skin lesions (< 1 mm of diameter) in nodular melanomas and *in situ* melanomas. For this reason a thermal stimulus, such as cooling-down, permits one to discriminate even a very small malignant tumor. Observation of temperature recovery up to the basal gradient, after the thermal stimulus has been removed, distinguishes benign from malignant lesions.

Di Carlo employed Thermovision camera 680 (AGA AKTIEBOLAG, Lindigo, Sweden) to analyze 402 melanomas both with and without thermal stimulation (5°C for 20 seconds). The author obtained a rate incidence of 23.4% false negatives without thermal stimulation and 4.4% false negatives applying thermal stimulation (27). The same procedure was significantly able to distinguish allergic reactions in contact dermatitis from irritant reactions (AGEMA Thermovision™, AGA Thermovision™ and FLIR 3000 Thermocam™), extending the method to various dermatological fields (28). Santa Cruz GA *et al.* (2009) adopted a different protocol of thermal stimulation in nodular melanoma patients treated with Boron Neutron Capture Therapy (BNCT). The procedure consisted in immersing the skin lesion in cold water (15°C for 2 minutes) or in application of an alcoholic spray. Cold stimulus causes vasoconstriction in normal vessels and increments thermal contrast in malignant areas. The measurement of skin temperature for 3 minutes (Rayton Palm IR 250, L3 Comm. Systems) revealed an augmentation of 6-10°C in nodular lesions compared to surrounding regions (13, 26). Herman C and Cetingul MP applied cold air by a vortex tube (5 minutes), removed the thermal stimulus for 1 minute, and then measured the temperature (Merlin infrared camera MWIR) (29). The authors consider that the first recovery phases of temperature, after thermal stimulation is removed, are the most important in dis-

tinguishing malignant melanoma from benign lesions and healthy skin: melanoma showed temperatures from 21.5°C and 19.2°C after 30 seconds and from 24.9°C and 22.7°C after 60 seconds, while benign lesions and healthy skin have the same pattern (0.5°C temperature variation after 60 seconds) (30).

The aim of our project was to evaluate the efficacy of thermography and dermatoscopy in the screening of skin lesions. The results showed 60.87% of hot lesions. Although the datum is not significant, it suggests the existence of a metabolic mechanism in the skin, which is detectable by thermograph. Moreover the protocol is a valid means to monitor the evolution of skin lesions, especially in young people. On average the temperature of skin lesions was higher than that of control lesions (0.67°C; Table 1 and Figure 9). This result supports the existence of morphologically atypical nevi. Our result concerning the mean recovery of temperature after cooling down (+ 2°C in 30 seconds) is comparable with findings obtained by Cetingul *et al.* (30). However it is difficult to express more considerations about this topic because of the scarceness of scientific data.

The histograms in Figure 10 and Figure 11 show that feet, genital organs and arms are hotter in 100% of cases; while in the back, abdomen, breast, face and neck there are no significant differences. In the legs we found a greater number of non-hot lesions compared to hotter lesions (27.78% *vs* 7.14%). The results suggest to monitoring feet, where lentigo melanoma spreads (31), the genital area, where melanoma is associated with other cutaneous melanomas, and to familial predisposition (32), and arms.

Photo-type 2 (light skin, elevated UV sensitivity, blue/green eyes, blond hair, freckles) is more frequent in our sample (Figure 6). The fact suggests greater susceptibility to developing skin lesions in subjects having less quantity of pigment cell, pigment which is a barrier to UV exposure. It absorbs and repulses parts of UV, and neutralizes free-radicals forming after UV mechanism stimulation (33).

Oncologic risk assessment (Figure 7) suggested the existence of a low-medium risk (N:2-3). This result supported the thermal results and suggested monitoring the morphological evolution of skin lesions, and/or to carrying out a histological examination.

Total morphologically atypical nevi are distributed especially in the back, face and neck (Figure 8). This result agrees with previous researches and supports the thesis that the body areas with most risk of developing melanoma are the back, face, neck, legs and arms (34, 35). In particular the arms are hot body areas in 100% of cases in our study.

We have reported only three cases of personal oncologic history (thyroid gland carcinoma, Hodgkin's and non-Hodgkin's lymphoma) and data relating to oncologic familial history (Figure 12). These results enable us to assess the risk of young people to developing melanoma. In certain melanomas some events exist such as CDKN2A and BRCA2 mutations in women with a breast cancer history, similar to mutations in breast cancer. Consequently it is evident that women healed from breast cancer are more exposed to develop melanoma and *vice versa* (36). BRAF mutations identified in melanoma and in thyroid gland carcinoma suggest a common pathogenetic mechanism in both malignancies (37). Moreover TSH and MSH hormones stimulate both melanocyte and thyroid gland cells to synthesize cAMP in BRAF signaling. So it is evident that TSH and MSH pathway involved both pathologies (37). Finally subjects surviving melanoma have a higher risk (OR=1.57) of developing non-Hodgkin's Lymphoma (38).

The results on familial old age are uniform both in selected patients and in the total group (Figure 13 and Figure 14), and an exactly alike mean age to that of the patients' parents older than 90 (94 years old) exists.

The poor thermal results obtained may be attributed to the moderate sensitivity and resolution of the thermograph. However it is probable that by incrementing sensitivity and accuracy we can obtain significant diagnostic results. We expect to extend follow-up at least for 10 years, after having educated patients to identify symptoms related to malignant transformation. In fact during this period subjects will produce documentation allowing us to make an early diagnosis.

Conclusions

The conclusions of the present work suggest applying an innovative screening protocol on a healthy

young population, sensitive to the theme of early cancer diagnosis. Tele-medicine may be a low-cost and efficacious follow-up means to devise surveillance data banks. Patients were enrolled by a low-cost merchant group (Groupon) that continually recruits new clients. Our research introduced the integration of dermatoscopy by an Optilia instrument and thermal camer Flir i40 to formulate a reliable diagnosis, based on morphological characteristics of skin lesions detected by the two instruments. Finally, the validity check on the present study largely depends on the screening performed by technological instruments but it is also related to histological examination.

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Flir Systems Srl, Limbiate (MI);
Cortex Technology ApS, Hadsund, Denmark.

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