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RESEARCH ARTICLE

A Preliminary Study for Quantitative Assessment with HFUS (High-Frequency Ultrasound) of Nodular Skin Melanoma Breslow Thickness in Adults Before Surgery: Interdisciplinary Team Experience

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Abstract: ***Background:*** Cutaneous melanoma is one of the most severe skin diseases. Nodular melanoma is the second melanoma subtype in order of frequency. The prognosis of skin melanoma depends on the vertical growth of the tumor (Breslow index). For this measurement, excisional biopsy is strongly recommended. This is, however, an invasive procedure and may cause damage to the lymphatic drainage system. The HFUS system, , can be extremely useful for determining tumor thickness in the preoperative phase, given its high resolution capacity. The aim of this preliminary study is to define the role of HFUS for the nodular skin melanoma Breslow thickness in adults before surgery by making a comparison with histological features.

Methods: In this study, 14 melanocytic lesions (8 male and 6 female) were evaluated with dermatoscopic clinical features strongly indicative of nodular melanoma. Out of these, excisional biopsy of 7 lesions was requested. The ultrasounds were performed preoperatively. The images were acquired through the first ultrasound scanner with ultra-high frequency probes (range from 50MHz to 70 MHz) available on the market under the EEC mark (Vevo "MD, FUJIFILM Visual Sonics, Amsterdam, the Netherlands) equipped with a linear probe of 50-70 MHz.

Results: From the ultrasonographic analysis of 14 nodular melanoma thickness was determined for the presence of two hyperechogenic laminae, separated by a hypo / anechoic space. The twelve lesions were in situ while the other two lesions showed ultrasonography for example; the satellite lesions (less than two centimeters from the primary lesion) and in transit (localizable to more than two centimeters from the primary lesion). Four of these lesions were ulcerated. A comparsion was made the 7 lesions on between the thickness calculated with this method, and that obtained on the bioptic piece. The presence of a positive concordance has been evident in all of the cases.

Conclusion: If further studies are needed to support its widespread clinical use, its is believed that, in expert hands and with an interdisciplinary team, HFUS is already capable to reliably calculate a Breslow index in a large majority of patients with cutaneous melanoma.

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1. INTRODUCTION

Over the last twenty years, the incidence of malignant skin melanoma and its mortality has increased. The global incidence is approximately 160,000 new cases every year with 48,000 deaths [1-3]. The most important prognostic factors are the vertical growth of the tumor (pathologic Breslow index) and the invasion of the dermis. The thickness of the lesion is a fundamental oncologic benchmark; one millimeter thickness defines a favorable prognosis for survival while the lesions with greater thickness (>2mm) have a poor prognosis. The poor prognosis also correlates with in-

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creasing age, male sex, and localization (truncal and head and neck locations have a poorer prognosis than tumors on the limbs). This index is positively related to the probability of lymph node involvement and with the risk of distant metastasis. The histologic prognostic factors are the presence of ulceration, mitotic rate (number of mitoses/mm^2), and Clark level of invasion. Tumor thickness is one of the most important factors in determining surgical strategy in the treatment of melanoma [4-5]. The principal factors determining surgical strategy in melanoma treatment include tumor thickness (Breslow index), grade (Clark level), ulceration, and regression. The measurements of skin thickness preoperatively are important to establish the correct therapeutic approach. HFUS increases and supports the other methods. The better resolution of the high-frequency imaging improves the diagnostic value of the ultrasounds to determine the lateral margins and the thickness of the lesion. Highly accurate preoperative evaluation of cutaneous melanoma lesions is essential for establishing an optimal therapeutic approach and for improving the survival rate. The use of very-high-frequency ultrasound (> 20 MHz) increases the accuracy in differentiation of the skin layers, improves characterization of skin lesions, and increases the accuracy of measurement of the thickness of the lesion. Surgical wide excision continues to be the cornerstone in the treatment of primary melanoma. For thin melanomas (Breslow index ≤ 1 mm), a circumferential radial margin of 1 cm is the standard margin in most clinical guidelines. For tumors with a Breslow index of > 1 mm and for ulcerated tumors or Clark level IV tumors regardless of Breslow index, standard treatment consists of excision with expanded safety margins of 2 cm and concomitant sentinel lymph node biopsy [6-8]. The histologic status of the sentinel lymph node is the main predictor of prognosis and survival in clinically node-negative melanoma patients. In its most recent version, the American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma considers sentinel lymph node biopsy results along with Breslow index and the presence or absence of ulceration of the primary tumor. If tumor thickness could be accurately estimated before biopsy, tumors with a Breslow index of ≤ 1 mm could possibly be resected with appropriate safety margins in a single surgery. For tumors with an estimated Breslow thickness of > 1 mm, patients could be offered the option of having a single surgical procedure with both sentinel lymph node biopsy and wide-margin excision of the tumor. In most studies evaluating sonography for measuring cutaneous lesions in the published literature, investigators used frequencies of ≥ 20 MHz [9-14]. For the current study, the possibility of using 70-MHz sonography was evaluated to measure nodular melanoma thickness before surgical excision. Nodular melanoma is the second melanoma subtype in order of frequency. It is characterized by a pigmented lesion detected at a rapid growth (even of a few weeks), often accompanied by ulceration and bleeding**.** The recent advances in ultrasound technology have allowed the observation of the cutaneous layers with good resolution. The literature has sequentially increased sonographic information about primary cutaneous melanoma lesions [15-17]. The aim of this preliminary study is to define the role of HFUS for the nodular skin melanoma Breslow thickness in adults before surgery by making a comparison with histological features.

2. METHODS

In this study, the ability of ultra high frequency ultrasound waves was evaluated to provide more useful information in the pre-excision phase for nodular melanoma. The images were acquired through the first ultrasound scanner with ultra-high frequency probes available on the market under the EEC mark (Vevo "MD, FUJIFILM Visual Sonics, Amsterdam, the Netherlands) equipped with a linear probe of 50-70 MHz.

The experimental protocol (DR/04 - 01/2018) was approved by the Institutional Ethics Committee of Campania University “Luigi Vanvitelli”, Naples, Italy.

A copious amount of gel was applied to the surface of the lesion, without using a spacer or other devices. During the study, 14 melanocytic lesions (8 male, 6 female) were selected and analyzed with dermatoscopic clinical features which were strongly indicative of nodular melanoma.Excisional biopsy was requested. HFUS was performed preoperatively, before excision. The probe used was HFUS (70 MHz), which has the ability to select frequencies independently on the basis of the structure to be analyzed. Every lesion was analyzed in terms of the morphology, the size, the presence of satellite and transit metastases, and the relative vascularization (using Eco-Color Doppler). In order to analyze the affected nodular skin melanoma, it was important to avoid any form of cutaneous compression or flattening of the lesion. Therefore, a copious amount of gel was used to separate the probe from the skin. A flattening of the lesion caused by excessive compression can cause an altered thickness measurement. For every lesion, a systematic study was performed of the skin around the primary lesion. An area of at least 10 cm in diameter in the location of the lesion was analyzed, especially in the direction of the local-regional drainage lymphatic stations. In this way, it was possible to obtain a panoramic exploration of an extended part of the body (for example of an entire limb). The HFUS data were compared with histopathological (AP).

3. RESULTS

During this study, from ultra-sonographic analysis, 14 melanocytic lesions were prospectively evaluated with dermatoscopic clinical features which were strongly indicative of nodular melanoma and required an excisional biopsy. . Seven lesions were located on the chest, four on the back and three on the leg. The ultrasound scans performed before excision demonstrate nodular melanoma, in whose thickness, the presence of two hyperechogenic laminae, separated by a hypo / anechoic space was appreciated. All the lesions were analyzed with 50-70 MHz probes to allow the study of the dermis and hypodermis. The result was well defined. The morphological aspects were the oval or fusiform shape, inhomogeneous, hypoechoic, with smooth edges and a variable degree of vascularization, easily appreciable at the Eco Color Doppler (Fig. **1a,b**).

In four out of fourteen cases, the imaging results consisted of ulceration namely the epidermis which was was irregular and discontinuous. It is possible to appreciate an increased echogenicity in the subcutaneous tissue. In all analyzed nodular melanomas, the presence of two hyperechoic

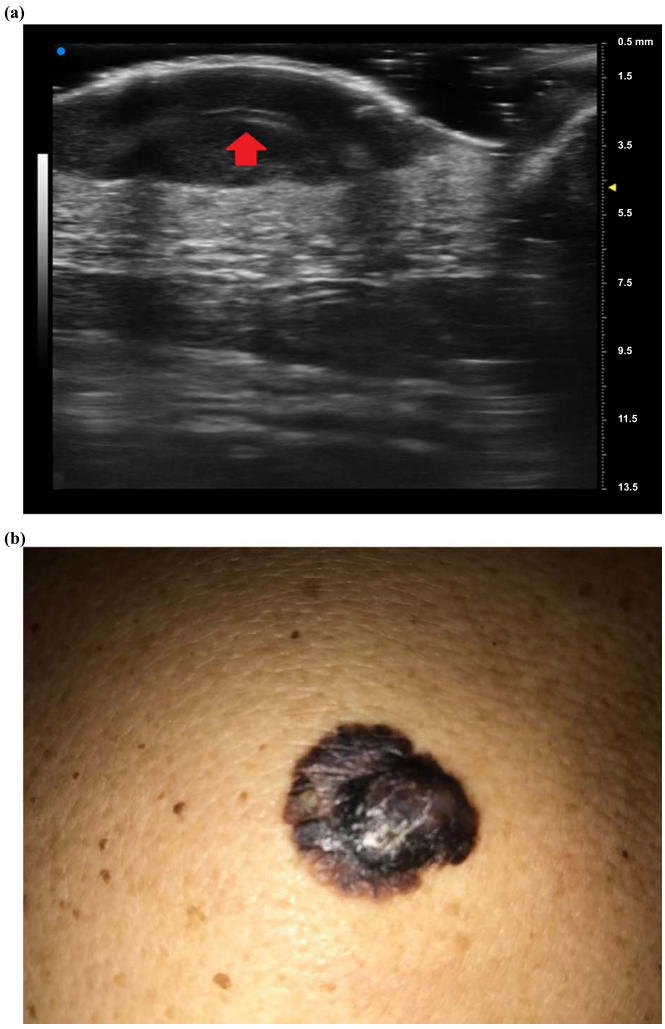


Fig. (1a,b). (**a**) left image shows the example of the nodular skin melanoma evaluated with HFUS (50 MHZ); (**b**) the image shows the same nodular melanoma evalues with dermatoscopic approach.

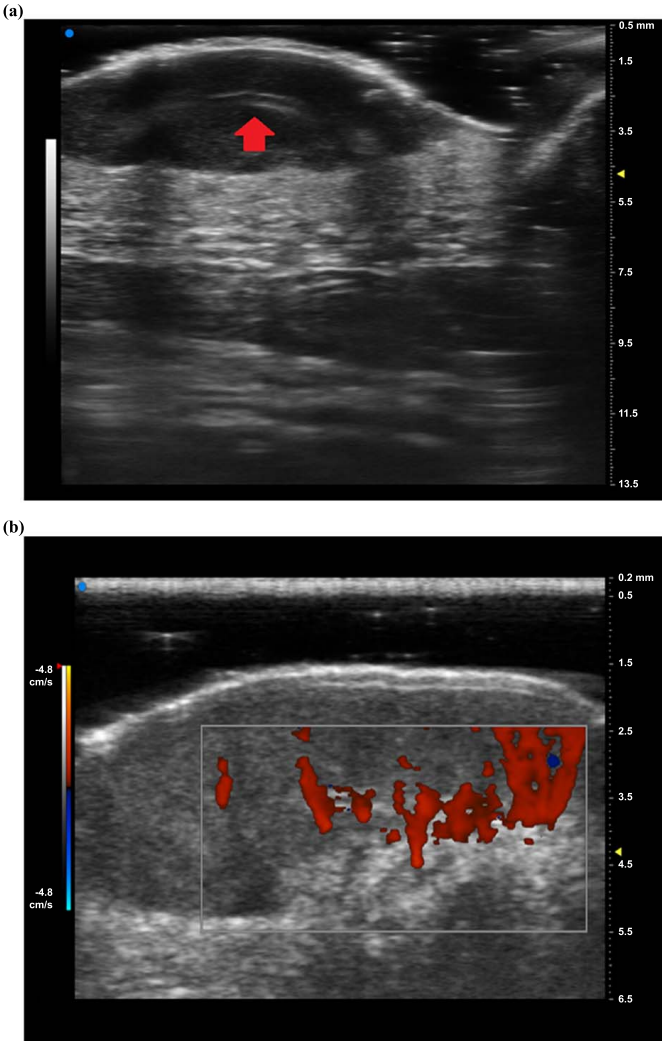


Fig. (2a,b). Example of the nodular melanoma with hyperechoic stria inside this structure is probably a neo-vascular tumor formation **a**) the left image shows the nodular melanoma acquired with 50MHz probe;the right image **b**) shows the increasing of the signal of the Duplex-Doppler.

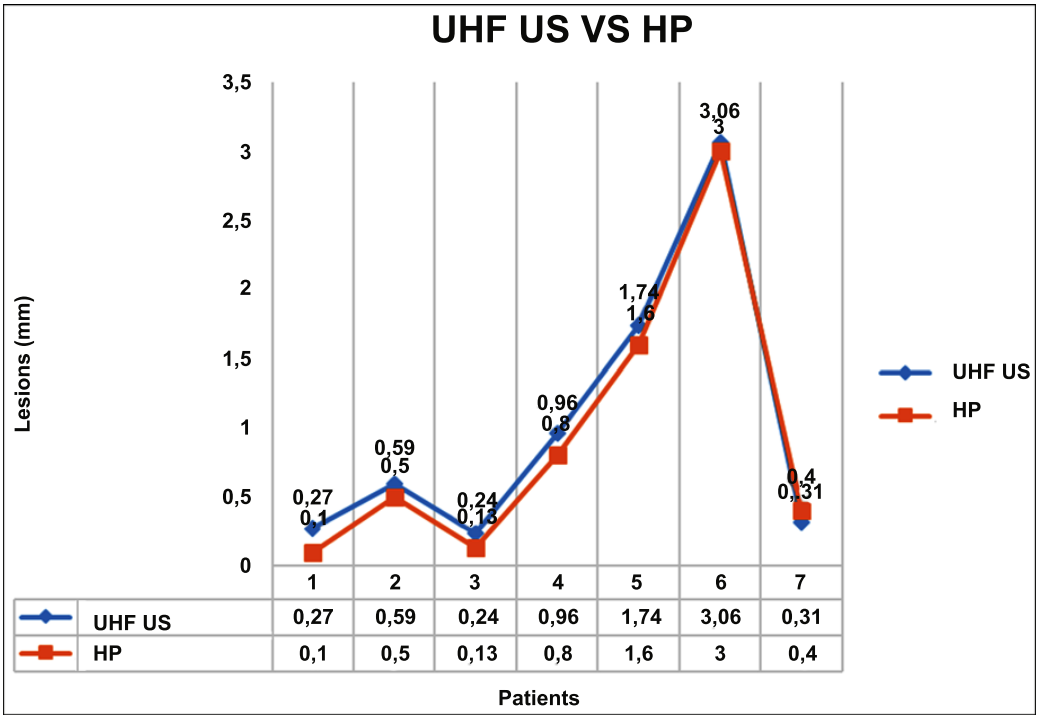
laminae, separated by a hypo/anechogenic space was observed. This structure is probably a neo-vascular tumor formation. This hypothesis is also supported by the information obtained from the Eco Color Doppler (Fig. **2a,b**).

Furthermore, twelve out of the fourteen lesions were in "situ" nodular melanoma. The imaging results showed the presence of a regular hyperechoic band that represents the border point between the lesion and the dermis**.** This structure is probably indicative of the phase of radial growth of the lesion, in which the cells have not yet acquired the ability to invade the deeper layers of the skin. The other two lesions shown by imaging satellite lesions (less than two centimeters from the primary lesion) and in transit (localizable to more than two centimeters from the primary lesion) are hypoechoic, oval or round shaped, smooth or lobulated margins with variable degrees of heterogeneity and vascularization. The comparison between the thickness calculated with this method, and that obtained on the bioptic piece, has shown in all cases the presence of a positive concordance (Scheme **1**).

4. DISCUSSION

After years of studies on small animals, it was possible to strengthen the ultrasound technique for clinical use by introducing the first HFUS on the market [18]. The HFUS is widely used in the preclinical imaging on the animal model above all to study the oncologic lesions like skin melanoma and the cardiovascular system.

The instrument of this study uses frequencies between 50-70 MHz, which are much higher than the conventional ultrasound systems available until now in the clinical practice, providing a resolution of up to 30 microns and a penetration of about 15 mm [19-22]. HFUS ultrasound can be



Scheme 1. The comparison between the thickness calculated with this method, and that obtained on the bioptic piece, has shown in some cases the presence of a positive concordance.

considered the future of ultrasound imaging due to its improved spatial resolution despite the reduction of penetration depth. . It is, therefore, a technology with great potential and with great impact on the medical field.

This system is suitable for a series of specific clinical applications with the possibility of carrying out various types of examinations, including: abdominal, pediatric; neonatology; of small organs (breast, thyroid, testicles, prostate); Musculoskeletal (conventional and superficial); of peripheral vessels; dermatology. Indications include imaging for positioning needles and catheters within vessels or other anatomical structures, imaging for eco-guided procedures of peripheral nerve blocks [23-27]. The use of HFUS transducers, on the other hand, limits the depth of the images.

The transducers developed till now are of three types and each provides a maximum imaging depth: HFUS 22 Mega-Hertz (MHz) = 38.4 mm, HFUS 48 (MHz) = 23.5 mm, HFUS 70 (MHz) = 10 mm. The use of the system depends on the type of transducer used.

The imaging of the skin layers is often approximate and limited to the conventional ultrasound.

Instead, this system presents an excellent dermatological application, allowing an evaluation of:

- Skin and subcutaneous layers

- hair follicles

- identification of foreign bodies

HFUS may have a complementary role in physical examination in the evaluation of superficial skin lesions that are "too small" to be analyzed with conventional ultrasound. In particular they can be used in the evaluation of skin layers and in assessing the subcutaneous involvement of neoplastic lesions, in order to reduce the percentage of diagnostic error and recourse invasive procedures such as biopsies or aspirated needles [24-29].

HFUS applied to the study of the mammary region could provide detailed information about the anatomy of the premature area on the superficial skin layer and on the retroareolar tissue, finding an indication for example in women presenting mammary secretions and give a hint in the differential diagnosis between benign and malignant neoplasms, inflammatory diseases and infectious diseases. The first sonographic reports on melanoma were performed using other types of ultrasound equipment that used fixed high-frequency probes that are between 20 to 100MHz. The latter results may perhaps be related to the low penetration of these fixed frequency ultrasound machines (approximately 6mm at 20MHz, 3mm at 75MHz, and 1mm at 100 MZ). This point could be relevant since the skin presents a variable thickness of its layers according to the corporal region; thus, only the dermis could measure more than 3.0 mm (thickness) in normal individuals for example, in the dorsal thoracic region [29-32]. Thus, the recognition of vital information can support the performance of “one-time” surgery in melanoma, where the size of the incision and the free margins with the sentinel lymph node procedure are planned with imaging support pre-surgically. The use of sonography as a “thickness discriminator” has shown high correlation with histological results [33-35]. Nevertheless, sonographic measurements can be slightly higher than histology because they correspond to *in vivo* tissue without dehydration or fixation, and may include sub-tumoral mononuclear infiltrate and a

nest of nevus cells which cannot be differentiated sonographically from melanoma cells [10]. This small difference in the measurements has been reported to be much less than the actual size of the extension used for assessing the free margins that is usually used in melanoma surgery. In this preliminary study, the comparison between the thickness calculated with HFUS, and that obtained on the biopsy piece, analyzed the presence of a positive concordance which was highlighted in all cases. Of the fourteen lesions analyzed, it was possible to calculate the dimensions and compare them with the results obtained on the biopsy piece. In the evaluation of superficial neoplasms, it can be used for thickness measurement, invasion depth, margin evaluation of tumors and post-surgical follow-up [36-39]. During sonography, early lesions usually present similar characteristics like oval or fusiform shape and hypoechogenicity. Commonly, these lesions infiltrate the dermis and show increased blood flow within the tumor. Since the tumor can show asymmetry in its shape, the measurement of thickness should be performed at the deepest point. In one case with ulceration, the epidermis was irregular or discontinuous, and increasing echogenicity of the surrounding subcutaneous tissue was observed [40-45]. The nodular melanoma skin lesions required the use of a copious amount of gel on the skin surface to obtain reproducible image and the exact measurement of the thickness. The use of a copious amount of gel it is also recommended to avoid the compression of the vessels that show low velocity, especially in bulky tumors [46-50]. In two out of fourteen cases, local metastases was observed because of Duplex-Doppler analysis. Among the settings, the power Doppler that is usually more sensitive for detecting slow flow could be more useful than the color Doppler mode [51-52]. The peripheral perfusion has been mentioned as an early sign of involvement and it is of crucial importance to achieve a high identification rate of metastasis. Balloon shape and loss of central echoes are indeed late signs of malignant nodal infiltration. Since the work by Srisvastava *et al*. was published in 1989, several studies have focused on the analysis of tumor microvasculature. The detection of microvessels in a skin lesion increases the conspicuity for malignancy, but reports in the literature are still variable because microvessel detection has shown a good specificity for malignancy (90%–100%) but sensitivity has been in the 34%–100% range. These variations could be related to the natural history of angiogenesis in tumors and also with the advances in ultrasound [52-54]. A relevant advantage of HFUS in the preoperative phase is the evaluation of the lymphatic system. For this measurement, excisional biopsy is strongly recommended. This is, however, an invasive procedure and may result in damage to the lymphatic drainage system. The high-frequency ultrasound system, given its high-resolution capacity, can be extremely useful for determining lymph node involvement. The results of this study demonstrate a positive correlation between ultra high-frequency ultrasound and histopathological examinations. [55-60] This is a preliminary study and data should be confirmed on a larger number of patients with nodular melanoma. While keeping this reserve in mind, these first results are very encouraging. The method will be verified to examine if it can be used to obtain an ultrasound staging ("sonographic T"), which may be important in predicting a possible lymph node involvement and reducing the need for reoperation to consolidate the excision-free margins of pathology. Thanks to the advanced technologies. It will be possible to study tumor angiogenesis using the hybrid technique ultrasound and laser in one probe.

CONCLUSION

If further studies are needed to support its widespread clinical use, it is believed that, in expert hands, HFUS is capable to reliably calculate a Breslow index in the large majority of patients with cutaneous melanoma. HFUS could represent a preliminary method before surgery to define the correct treatment. It could also be useful as a guide to superficial lesion biopsies in monitoring the effects of topical and systemic drugs. The ultrasounds could progressively become the most powerful support in diagnostic procedures [39-44]. The HFUS is not intended to replace histology but perhaps the anatomical information can provide a missing link between clinical evaluation, biopsy and treatments.

LIST OF ABBREVIATIONS

AJCC	=	American Joint Committee on Cancer
HFUS	=	High-Frequency Ultrasound
MHz	=	MegaHertz

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was approved by the Institutional Ethics Committee of Campania University "Luigi Vanvitelli", Naples, Italy, (DR/04 - 01/2018).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931).

CONSENT FOR PUBLICATION

A written informed consent was obtained from the patient prior to the study.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author, [AR], on reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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