Masterarbeit

Correlation between the number of melanocytic naevi on the face and total number of naevi.

eingereicht von
Gustavo Zanin Poletto

zur Erlangung des akademischen Grades MSc in Dermoscopy and Preventive Dermatooncology an der Medizinischen Universität Graz

ausgeführt im Rahmen des Universitätslehrgangs Master of Science in Dermoscopy and Preventive Dermatooncology unter Anleitung von Professor Iris Zalaudek, MD.

Graz, Österreich August, 2019

Declaration of Authorship:

I hereby declare that the thesis submitted is my own unaided work. All direct or indirect sources used are acknowledged as references.

Gustavo Zanin Poletto, São Miguel do Oeste, SC-Brasil 17/12/2019

DEDICATION

I dedicate my thesis work to all my family that always support me on all my endevours.

ACKNOWLEDGMENTS

First I would like to thank my supervisor, Professor Iris Zalaudek, for all the effort and time she spent helping me on this thesis, I know how busy her days are, between all her activities with the residents, research, and the International Dermoscopy Society, and I am really, really grateful for her time and knowledge.

Also, I want to thank all the group of dermoscopy from the University of Graz for this opportunity, and specially, for all the learning I have during all this time; I can say for sure that this knowledge already saved several patients and reduced morbidity markedly, by improving early melanoma diagnosis.

Last but not least I want to thank my wife, and all my family, for always supporting me, and making all the possible to help me reach my goals.

Gustavo Zanin Poletto

Medizinischen Universität Graz

Table of Contents

List of Tables

List of Figures

List of Abbreviations

BMI	Body mass index
BRAF	V-raf murine sarcoma viral oncogene homolog B1
C	Cytosine
CT	Cytosine-Thymine
CCTT	Cytosine-Cytosine-Thymine-Thymine
CPD	Cyclobutane pyrimidine dimers
CSD	Chronically sun-damaged skin
DOPA	Dihydroxyphenylalanine
DQ	Dopaquinone
DHI	Dihydroxyindole
DHICA	Dihydroxyindole-2-carboxylic acid
DNA	Deoxyribonucleic acid
ETBR	Endothelin B receptor
ET1	Endothelin-1
IARC	International Agency for Research on Cancer
IRF4	Interferon regulatory fator 4
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MSH-MC1R-CAMP	Melanocyte stimulating hormone - melanocortin 1 receptor - adenylyl cyclase pathway
MC1R	Melanocortin 1 Receptor
NRAS	Neuroblastoma-Rat Sarcoma gene
PET	Positron Emission Tomography
PTEN	Phosphatase and tensin homologue
PGE2	Prostaglandin E2
RAS	Rat Sarcoma
ROC	Receiver operating characteristics curve
ROS	Reactive oxygen species
SONIC	Study of Nevi in Children
SEER	National Cancer Institute's Surveillance, Epidemiology, and End Results

SNP	Single nucleotide polymorphisms
T	Thymine
TBSE	Total body skin examination
T1	Tumor size 1
T2	Tumor size 2
T3	Tumor size 3
T4	Tumor size 4
UV	Ultraviolet radiation
UVA	Ultraviolet radiation type A
UVB	Ultraviolet radiation type B
UVC	Ultraviolet radiation type C
UVR	Ultraviolet radiation
UK	United Kindom
USPSTF	United States Preventive Services Task Force

1. INTRODUCTION

In Europe the vast majority of deaths (close to 90% of the total) are due to noncommunicable (non-infectious or non-transmissible). Cardiovascular disease is the most common cause of deaths followed by cancer, with a estimated 1.75 million deaths from cancer in Europe, in the year 2012. (1).

Mortality rates around the world have been decreasing on four of the five cancers most easily accessible to early detection (skin, breast, cervix, colon, and prostate); with the exception of the skin, all of them, had mortality rate decline, over the last quarter century, ranging from 20 to 60% (2).

From all types of skin cancer melanoma is among the most life threatening due to its high tendency towards systemic metastasis. Although melanoma has a high chance of being curable (if detected early and treated promptly) mortality rates continue to rise (3-4).

Melanoma incidence has been rising in the past few decades among Caucasians. Indeed, melanoma is the malignancy with the highest rate of increase in incidence in this population, along with lung cancer in women (5-6). The individuals born in the United States of America in the year 2000 are under an estimated risk for developing an invasive melanoma of 1 in 75 during their life (7). In 2006 another study in the United States estimated that the risk for developing a melanoma was approximately 1 in 52 men and 1 in 77 women during their lifes. (8). This is a substancial increase when compared to the estimated lifetime risk of 1 in 1.500 persons in the 1930s (9).

Considering the fact that melanoma cure rates are directly linked to tumor thickness, and that invasive melanomas have a higher likelihood towards systemic metastasis, it becomes clear that careful patient screening to allow an early diagnosis is critical. A recently published study highlights the importance of early diagnosis, showing that ultra-thin (Breslow ≤0,5mm) melanomas have a ten-year survival rate of 99,6%, in comparsion to stage IIA melanomas, which have a five-year survival rate of 77-79%, or stage IV melanomas which have a five-year survival rate of 10% (10).

There are several risk factors considered relevant to melanoma development: genetic predisposition, exposure to ultraviolet radiation (UVR), number of sunburns during life, first degree relatives affected with melanoma, and also the total count of melanocytic naevi (11).

Over the last 20 years, several studies showed that host factors such as, high number of naevus and fair skin complexion are strongly associated with melanoma, and in fact are regarded as the most powerful markers of susceptibility for melanoma. In terms of phenotypical risk factors, the number and type of naevi are the most predictive features, followed by fair skin and eyes (12). The number of common melanocytic naevi, is regarded as one of the main markers of risk for melanoma in several studies (13, 14, 15). One study suggested that individuals with more than 25 common acquired naevi are twice more prone to develop melanoma than those with less than 25 naevi (16-17). Also, in individuals who already had developed a melanoma, the number of melanocytic naevi was considered the paramount phenotypic characteristic associated with the development of a second primary melanoma (18).

The effectiveness of melanoma treatment is directly dependent on the tumor size (it´s thickness) by, the moment of the diagnosis, as measured by the Breslow index (19-20). Usually melanomas present with a horizontal (or radial) and a vertical (perpendicular) growth phase; in general, the lesion begins in the epidermis and, with time, starts its vertical (deep) growth phase.

The total number of naevi and their histological subtype (for instance atypical naevi) are the most powerful risk factors for the development of melanoma in Caucasians, and the weight of the odds ratios (6-21) is greater than the odds ratios reported for UVR exposure and skin phototype (22).

Considering the aforementioned facts, and also considering the paramount importance of prompt detection of melanoma, it is surprising that full body skin exams are not routinely performed; not even by dermatologists, only 30% of whom routinely perform comprehensive skin examinations (21).

With these numbers in mind, the need for a simple and widely-available method for determining which patient is at highest risk of developing melanoma is apparent (23).

There are several researches that state that the number of naevi on the arms (not the forearm) to be a good prognosticator of the total naevi count, but this requires patients to unclothe, at least partially. Therefore, if the number of naevi on the face correlates with the total number of naevi, this could become a faster, easier and simple tool for the general practicioner to screen those patients who are in a greater risk for developing melanoma. Furthermore, a simple rule, such as, “More than 10 naevi on the face signifies a higher risk of melanoma,” can highlight the importance of the total number of naevi for non-dermatologists,

since many physicians are unaware of the direct correlation between the total number of melanocytic naevi and melanoma risk.

Limitations: Different results may be found in populations from other areas around the globe, and also with a different genetic background. All the individuals were examined in only one center, a private dermatologic clinic in the city of São Miguel do Oeste, Santa Catarina state, in southern Brazil. This region was mainly colonized by Italians, Germans and Polish imigrants (mostly fair-skinned individuals), and the majority of the population works as farmers, with chronic and intense sun exposure throughout their lives. Furthermore, most of the individuals are aged between 40 and 70. There may also be a sampling bias on the patient cohort, since it is a private dermatology clinic, so patients with a lower socioeconomic status may be underrepresented in the study population.

Hair color also may also correlate with the total nevus count, but in Brazil hair dying is very common among women, so it was decided to account for, in this study since the potential risk of patients misreporting their natural hair color could introduce a probable source of bias.

1.1 Melanocytes

Melanocytes are cell that emerge in the neural crest and in the course of the embryonic period migrate into the epidermis. They are responsible for producing melanin, a molecule that scatters and absorbs ultraviolet radiation (UV). The keratinocytes in the epidermis use melanin as a protection for their nuclei DNA against damage indued by UVR, rendering the melanocytes in the epidermis part of a defense system against UV radiation. The UV spectrum of sunlight is divided into three regions: UVA (320–400nm wavelength), UVB (290–320nm), and UVC (200–290nm).

Melanocyte precursors, during embryonic period, migrate (from their origin in the neural crest) through long distances until they reach the entire skin of the individual. This hability of melanocytes to travel long distances is one of the theories trying to explain the high metastatic potential that melanoma presents. Usually the neural crest is divided into four main functional domains: the cardiac, vagal, the cranial and truncal neural crest. Melanocyte predecessors travel from the truncal neural crest along the dorsolateral pathway through the dermis. The exact mechanism through which melanocytes migrate and stop in specific areas is

not fully comprehended and this is theorized to be influenced by the extracellular matrix and by signaling from the likely destinations (24).

Human skin has an average of 500-2000 melanocytes per square millimiter, each melanocyte, on average, supplies 36 keratinocytes with its melanosomes. Melanin production occurs inside the melanosomes and goes through the oxidation (via tyrosinase enzyme) of amino acid tyrosine to dihydroxyphenylalanine (DOPA) and to dopaquinone (DQ), it´s polymerization, and storage inside the melanosomes. Following this process fully developed melanosomes are carried via the dendritic processes of the cutaneous melanocytes and are finally transferred, via a mechanism that is barely understood, to contigous keratinocytes where they form a shield-like structure above their nuclei. Melanin act as a strong free radical hunter, and is also a robust antioxidant safeguarding the nuclei DNA from the damaging actions of UVR (25).

Skin complexion is not reliant on the quantity of melanocytes – their number is stable across the distinct skin phototypes- but rather on the amount of eumelanin (brown/black) and pheomelanin (red/yellow) synthesized by these melanocytes (26). The proportion among eumelanin and pheomelanin is paramount in creating the individual´s predisposition to suffer DNA damage through UVR, and consequently, tumor progression. Fair complexion individuals have propension 70 times higher to foster skin cancer in contrast to individuals with dark skin; confirming that there is a direct (and inverse) relationship between melanin content in the skin and incidence of skin cancer (meaning darker skin, lower skin cancer rates). The UVR sunscreen-like actions of melanin are mainly owed to eumelanin that acts to dissipate and absorb as much as 75% of UVR and, as well as, depletes the free radicals produced by the UVR, which in turn, protect the deeper dermis and tissues against UVR damage.

Pheomelanin is composed mostly of sulfur-containing benzothiazine and benzothiazole by-products (the main source of sulfur is L-Cysteine). Eumelanin, in opposition, is an utterly variegated polymer comprising of 5,6-dihydroxyindole (DHI) and or 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Melanogenesis results from the production and mixture of different proportions of eumelanin and pheomelanin; the rate of production is regulated by the substrate concentrations (tyrosine and cysteine) and the activity of the tyrosinase enzyme.

After being exposed to UVR, keratinocytes start to fabricate different cytokines and chemokines that impact melanin production. They, also, secrete large quantities of

prostaglandin E2 (PGE2), that exerts it´s effect by inducing the growth of dendrites in melanocytes (27).

UV radiation is one of the main environmental carcinogens, present in sun light and tanning bed; it is, also, associated on the genesys of melanoma, the most aggressive and deadly of all UV-induced skin malignancies. Accordind to Zaidi (2012):

> Terrestrial UVC is biologically irrelevant, as it is almost completely absorbed by the stratospheric ozone layer. Both UVA and UVB reach the earth's surface and have deleterious effects on nucleic acids and proteins. UVB is considered to be more carcinogenic than UVA, as it directly causes 2 types of DNA lesions: cyclobutane pyrimidine dimers (CPD), formed between adjacent thymine (T) or cytosine (C) residues, and 6-pyrimidine 4-pyrimidone photoproducts. The CPDs are more abundant, more carcinogenic, and less efficiently repaired. These UVB-induced lesions give rise to DNA mutations hallmarked by CT and CCTT transitions, the so-called "UVB signature mutations". On the other hand, UVA mutates DNA indirectly, thought to be mediated through generation of reactive oxygen species via absorption by endogenous photosensitizers (28).

Ultraviolet light, can be considered a through carcinogen since it operates both as a starter (initiator) and a booster (promoter). It induces immunosuppression and inflammation and it is thought that these actions combined result in DNA injuries, and in the emergence and/or development of melanoma (28).

Along the evolution many mechanisms arose to avoid, reduce, and correct the cellular alterations induced by UVR. These systems comprehend: rendering the epidermis thicker; the presence cytokeratin in suprabasal layers of keratinocytes; epidermal turnover of the squamous layers (the normal skin shedding); DNA repair activity; apoptosis of damaged cells; enhanced activity of antioxidant enzymes; and skin pigmentation (29).

Ultraviolet A rays (that comprise roughly 95% UV light that reaches the surface on the planet) has it´s carcinogenic action, mainly, via indirect injuries to the DNA through the formation of reactive oxygen species (ROS). On the other hand, UVB (which comprises less than 5% of UV spectrum) acts directly altering the DNA nucleotide structure as potent mutagen (30).

A 2009 study by Abdel-Malek et al. shed some light on how melanocytes deal with UV radiation exposure. What has emerged is that the UV resisteance of the melanocytes is coordinated by paracrine factors, most notably, the MSH-MC1R-cAMP (Melanocyte

Stimulating Hormone -Melanocortin 1 Receptor- adenylyl cyclase pathway) signalling cascade. They added endothelin-1 (ET1) and endothelin B receptor (ETBR) signalling as distinct system through which melanocytes guard themselves against UVR injuries. This could, in the future, become a promising new pathway for the prevention of melanoma (31).

In 2015 von Koschembahr et al. discovered lower leves of photodimers in melanocytes pretreated with ET1 before UV exposure. The protective effect by ET1 was measured, also, promptly after UV exposure, leading to the conclusion that ET1 may prevent the accumulation of UV photodamage in melanocytes, even before repair start. The pretreatment with ET1 reduced UV damage (based on cyclopyrimidine dimer load) by nearly 40% and, also reduced, even more the UV-mediated apoptosis, corroborating the hypothesis that ET1 enhances melanocytic resistance to UV injury (32).

On the other hand, studies showed that mice with mutations that activate BRAF are prone to a small percentage of spontaneous melanoma emergence; but when another mutation that inactivates MC1R is present, (in which eumelanin is not any more synthesized and results in red fur, with high contents of pheomelanin) there is a growth in the rate of spontaneous melanoma. Since in this model there is no UVR influence, the mechanism of increased risk for melanoma was theorized to be owed to an increased oxidative injury occasioned by pheomelanin. When the tyrosinase gene in these mice is deleted (there is a loss of pheomelanin synthesis) and the propension to melanoma is abolished, even in the presence of mutations activating BRAF and inactivating MC1R. This points to the conclusion that, the existence of pheomelanin is greatly active in the pathogenesis of melanoma (33).

There are some evidences that show that the synthesis of pheomelanin may be linked to an increased production of ROS (Reactive Oxigen Species). The process by which pheomelanin is synthesized requires big quantities of antioxidants, which leads to a depletion of glutathione (one of the scavengers of ROS) rendering the melanocytes more prone to ROS-related damage. Pheomelanin production requires cysteine which is delivered by glutathione. The pheomelanin production mechanism can, therefore, reduce the levels of glutathione and render melanocytes more susceptible genetic instability and to DNA damage. Research showed that oxidative stress is correlated with pheomelanin and glutathione depletion. It is theorized that the ionization potential in pheomelanin's aromatic ring´s sulfur content lowers, rendering it less balanced, and more potent for free radical formation when compared to eumelanin (34).

Melanosomes containing eumelanin are usually big and elongated, wihle the melanosomes containing pheomelanin are usually small and oval. There are evidences showing that pheomelanin is, also, involved in the preparation of the melanosome for the synthesis of eumelanin, and that, may have a role as a template, or a model, for the polymerization and deposition of eumelanin. The geographic distribution with an increased number of fair skin complexion individuals (blonde or red-haired) at greater latitudes advocates that the prevalence of pheomelanin results in increased UVR penetration (compared to eumelanin), allowing a more active metabolism of circulating precursors in the skin, which is a fundamental step in the synthesis of vitamin D.

1.2 NAEVI

Melanocytic naevi are benign neoplasms or hamartomas of melanocytes. Naevi may be congenital, (as standardized present at birth), though most are not evident until the first months of life. Considering the ample spectrum of melanocytic neoplasms and the intricateness of developmental and regenerative melanocytic pathways, several distinct processes may be involved in nevogenesis, and three basic proposed models involve epidermal melanocytic precursors, dermal precursors, and circulating precursors (35).

The clinical characteristics of melanocytic naevi are heterogeneous, and depend on the time during a person's life that the naevus is acquired, the specific differentiation state of the cell of origin, and their acquired genetic mutations. They may be classified in two major groups: congenital and acquired melanocytic naevi.

Congenital naevi are present in 1% to 3% of neonates at birth (or shortly after), and are divided in three categories based on their size (small <2cm, medium 2-20cm, and giant >20 cm), although this classification is subject to controversy. Congenital naevi usually present with a globular pattern on dermoscopy, terminal hair follicles, and often marked periadnexal downgrowth of melanocytes.

Acquired melanocytic naevi, which comprise the focus of this study, usually appear early in childhood; their number raises with age, and have a peak of incidence around the fourth and fifth decades of life. From the fifth decade onwards, there is a successive decrease

in the total number of naevi, as stated by the cliché, “we are born without naevi, and we will die without them” (36). Clinically, they are flat, superficial, and horizontally oriented lesions that are usually smaller (<6mm in diameter), than congenital naevi. Under dermoscopic exame, they usually present with a reticular, globular, or homogeneous pattern, alone or in combination. These distinct patterns reflect different arrangements of the nests. On the histology, melanocytes in acquired naevi are usually are monomorphous (small and oval-shaped) and do not involve the reticular dermis or the subcutis (37).

Factors predisposing to the development of acquired melanocytic naevi are heredity, childhood sun exposure (especially intense and intermittent), and skin phototype (38). Sunscreen use may be a protective factor (39). In a study by Moreno et al., there was a positive correlation between the number of melanocytic naevi and sun exposure, mainly in terms of the amount of time spent at beaches and pools, as opposed to other forms of outdoor exposure. Another study also supports the idea that intermittent and severe sun exposure (typically encountered during vacations) instead of chronic daily sun exposure, is the main cause of the number of naevi present in adults. Furthermore, significant holiday (intense and intermittent) sun exposure is associated with larger naevi, suggestive of more proliferative melanocytes, and less with clinically atypical naevi. This corroborates the view that, at least in those susceptible individuals (susceptible phenotype) intermittent sun exposure is related with melanocyte proliferation (40).

1.2.1 Atypical Nevi

Atypical nevi, also knowns as dysplastic or Clark’s nevi where described in 1978 by Clark et al. when they were detected in a family in which 36% had a diagnose of melanoma (41). In Clark et al., again, proposed five histological criteria for the characterization of a dysplastic nevus: lentiginous melanocytic hyperplasia, melanocytic nuclear atypia, lamellar fibroplasia, concentric eosinophilic fibroplasia, patchy lymphocytic infiltrate (42).

Atypical naevi are generally bigger than common naevi with a more assorted aspect, and is estimated tha 2–5% of Caucasian adults present them. The International Agency for Research on Cancer (IARC) developed a protocol for diagnosing and registering naevi in epidemiological studies utilizes the following criteria to identify atypical naevi: “there must be a macular component in at least one area; in addition, at least three of the following

features must be present: (a) border not well defined, (b) size 5mm or more, (c) colour variegated, (d) contour uneven, (e) presence of erythema".

Clinically atypical moles often have a peculiar appearance, typically, they are large pigmented lesions and generally measure between 5-15mm in diameter (thus bigger than commom nevi) and are asymmetric; several studies use the size above 6mm as a cuttof for classifying a lesion as atypical. Borders are usually irregular, ill-defined and notched. Color is widely variable, and goes from tan to dark brown to pink, and some lesions may, also, present elevated, or papular areas.

There is a higher prevalence in males (which is believed to be related to gender differences in UVR exposure). The prevalence of atypical nevi in fair skin populations goes from 2% to 8%, depending on the source (43) but, also, as high as 17% depending on the diagnostic criteria used (44). A meta-analysis of case-control studies showed that individuals with one atypical mole have a 1.45 relative risk of melanoma in comparsion to individuals with none; and this risk boosts to 6.36 in those individuals with five atypical moles (45).

1.2.2 Naevi numbers

Higher naevus counts are associated with lighter skin phototypes, and correlate also with the number of sunburns, especially during childhood. On average, a white child has 15-30 melanocytic naevi (46), although this number can vary greatly. A prospective study in Vancouver, Canada followed 164 white children (between the age of 6 and 10) for a period of 3 years, recored an increase in median whole-body naevus counts 68 to 96 naevi (47).

Another study found that the number of naevi among 7-year-old school children in Sweden has a marked north–south difference, with a markedly smaller quantity of naevi in northern Sweden children; the Swedish latitude spreads from 55 N to 69 N, with the temperate climate of southern extreme of Sweden diverging markedly from the cold arctic climate of northern Sweden (48).

In 1952, in a group of 1000 individual from an US outpatient service, the average mole count in was 14.6 lesions (49). In 1973, Nicholls, in Australia, evaluated 1,518 individuals and the peak incidence of nevi for females aged 20–29 years was 27; for males the peak incidence was at age 15 with an average of 43 nevi. (50).

Another study, also, from Sweden comparing body-site proportions of naevi in southern and northern Swedish children found they were very similar but, in consoance with

melanoma, a slightly bigger prevalence of truncal localisation in southern Sweden children, where the climate is sunnier. Naevi gender disparities matched the gender distribution of melanoma, in which males develop more naevi on the trunk and face while females have more on the lower extremities. The distribution of naevi in northern Sweden 7-year-old boys, also, matched with melanoma distribuition on the 30–49 and 50–69 years age groups. For the southern Sweden boys significant concordance was present with melanoma distributions in the 0–29, 20–49 and 50–69 years age gropus (51).

In the year 2000 a study from Naldi et al. found in the control group (composed of 538 patients without history of melanoma) that 76,6% had between 0 and 15 melanocytic nevi (52). Also, in the year 2000 Schäfer et al., evaluating 2823 adults found that 60.3% had between 11 and 50 common nevi, and in 8.1% more than 50 nevi were discovered on the physical examination (51).

According to several different studies men present more melanocytic nevi most probably because of higher sun exposure.

A high nevus count also is directely correlated with a incresead chance of a second primary melanoma (26).

The precise classification of what is considered a high number of nevi is wide, with several studies classifying as more than 50 nevi and others more than 100 nevi. For this research the cutoff of 50 nevi was utilized.

1.2.3 Nevi natural history

Naevi undergo dynamic turnover and changes during one's lifespan, as they may appear and disappear over time. This is supported by a longitudinal study from Queensland Australia, in which 20 adolescents aged 12 to 14 years had facial and neck naevi evaluated. After 3 years, from the 230 baseline naevi, 190 new naevi were observed and 61 (26.5%) disappeared (53). In the SONIC study, nevi that appeared at age 14 where followed through age 17 (n=121) a small percentage faded completely (5%), or shrink in size (2%), the majority either grew in size (43%) or remained stable (50%). Children with higher number of nevus on the back had greater naevus variability, being more prone to both develop new naevi and to have naevi disappear during the study period (54). This confirms the findings of a 1994 study of 2552 Australian school children aged between 5 to 14 years that focused on total-body naevus

counts. At the age of 5, the median total naevus counts (whole body) was 40 for girls and 51 for boys; these numbers increased over time with a count of 96 for girls and 120 for boys at the age of 14. (55). This dynamic period during childhood and adolescence may support the theory that excessive or intensive sun exposure during this period of life is one of the main enviromental components for the development of melanoma. Acquired melanocytic naevi are clinically apparent and generally benign, though potentially precancerous lesions. This may be compared with other precancerous lesions such as actinic keratoses, which appear later in life.

Fewer than 5% of naevi undergo any noticiable change when closely followed (56). On the other hand, it is estimated that up to half (25% to 50%) of all cutaneous melanomas emerge from a precursor melanocytic naevi. A meta-analysis by Lin et al. involving more than 4.000 cases (in 13 studies) revealed that 32% of melanomas present with a naevus associated (57).

According to Tsao et al., the annual transformation rate of any single naevus to melanoma varies from roughly 1 in 200.000 in individuals below the age of 40 years to about 1 in 33.000 in men above 60 years of age. Based on these numbers, during a life span, the odds for one specific naevus to evolve to melanoma is about 1 in 3.000 for men and 1 in 11.000 for women. This same study, also, discovered that half or more of melanomas of patients younger than 30 years are associated with a melanocytic naevus precursor, while percentage falls to roughly 20% for patients older than 60 (58).

Zalaudek et al. proposed a dual nevogeneis concept based on dermoscopic studies. Congenital, compound and dermal naevi originate early in life (or even in utero) and are mainly influenced by genetic factors. These naevi persist during life, rarely regress, and exhibit a globular pattern. On the other hand, junctional or acquired naevi are induced by enviromental factors, namely UV radiation, and increase in number, peaking in adult life, with later involution; histologically, these naevi show a reticular pattern (59). This concept states that whereas globular naevi develop through mutations involving c-kit, c-met, or N-ras, reticular naevi develop as the result of exogenous factors, specifically ultraviolet light exposure (60). This hypothesis is supported by two different studies, one from Italy and the other from the United States, that showed gradual reduction in the ratio of globular naevi and an increment in reticular naevi starting around de the age of 20 years (61). It must be noted that, from the dermoscopic perspective, the SONIC study found that most naevi keep a

constant dermoscopic pattern from baseline to follow-up evaluation, and in fewer than 2% of naevi a change of pattern between globular and reticular occurs (62).

Whiteman et al. based on the prevalence of melanomas on different anatomical areas proposed ‘‘divergent pathway’’ on sun exposure and naevi. Research showed that people with small quantities of melanocytic naevi, many solar keratoses, and have high levels of professional (job-related) sun exposure where more prone to develop melanomas on head and neck. On the other hand, individuals with several naevi, few solar keratoses, and lower levels of occupational sun exposure tended to have melanomas (of the same histological type) arising on the trunk. It is theorized that in individuals with high nevus count (nevus-prone phenotype) after initiation by sunlight, melanocytes start to proliferate and become neoplastic with little additional need for UVR exposure. Conversely, individuals with low nevus count (thus with a low propensity to develop naevi) need long and chronic exposure to UVR to stimulate the rise of melanoma, further than that required for initiation; in these group, melanomas have a tendency to develop on areas fo the body exposed do UVR and are associated with chronic sun exposure (63).

Many acquired melanocytic naevi, as well as, small and medium-sized congenital melanocytic naevi, may harbor a key mutation in the BRAF gene, that results in the change of glutamic acid for valine at position V600 of the protein within the kinase domain in exon 15, this change leads in a constitutively active BRAFV600E. BRAF is a serine-threonine kinase that is activated by the RAS family of proteins that, when activated, triggers the MAP kinase signaling cascade (MAPK) (64). Almost 80% of benign naevi carry the BRAFV600E mutation, which is also the most prevalent mutation in melanoma, detectable in about 60% to 70% of malignant melanomas (65), what may explain, at least partially, the connection between the number of nevus and the risk of developing melanoma. In the greater part of these benign neoplasms, tumor suppressor proteins like p16, activate melanocyte senescence, leading to growth arrest, rendering melanocytes stable or even leading them to involution. On the other hand, some individuals, develop a bigger number of nevi, and usually, the melanocytes keep proliferating for a longer period prior to the activation of senescence.

The naevus phenotype and the risk for melanoma are associated with single nucleotide polymorphisms (SNP) in interferon regulatory fator 4 (IRF4) located on chromosome 6 and further SNPs on chromosomes 9 and 22. The number of acquired naevi an individual develops over his life is strongly associated with SNPs of the chromosomes 9 and 22.

The location of the naevi is mainly truncal in younger children. Adolescents and adults share a similar pattern, with a propensity to develop naevi on the trunk followed by limbs. Also, the regional distribution of naevi follows different patters in men and women, with the former having more naevi on the trunk, while the latter have more naevi on the legs, in consonance, again, with the anatomic sites of highest incidence of melanoma in the two genders (66).

In fact, naevi and melanomas share almost the same enviromental and host risk factors such as UV exposure, specially, early in life (sunburns and chronic exposure during childhood and adolescence) as well as known genetic factors such as UV-induced mutations in oncogenes such as BRAF. As previously stated, BRAF is mutated in approximately 80% of naevi, and NRAS mutations have been found in 6-18% of naevi these two mutations, are, also, the most common mutations found on melanoma (67).

In the UK, in 22% of melanomas occurring before 40 years of age, the individual has a nevus count of 100 or more. With the progression of age, the total number of naevi reduces significantly and above the age of 40 years only 12% of melanomas are linked to high nevus counts. For all age gropus, fair skin (phototype 1 in the Fitzpatrick scale) responds for 9% of all melanomas in the UK (28).

Evidence suggest that, since naevi are clonal, the genesis of naevi in humans takes place as a result of only one single activating mutation in the mitogen-activated protein kinase (MAPK) pathway in one individual melanocyte (68).

According Damsky et al., after the BRAF mutation, MAPK pathway is only briefly activated and this reflects the stage of active melanocyte proliferation during naevus formation (that lasts from 1-12 months). When the naevus enter in the growth arrest phase, MAPK activity lowers significantly. The exact path by which MAPK signaling is mitigated during growth arrest phase in nevi is not well understood. Progression to melanoma seems to depend, at least partially, upon reactivation of MAPK signaling (70).

Also associated with growth arrest after oncogene inactivation are tumor supressors p16INK4A and p14ARF, the former, in particular, is highly upregulated in cells that have undergone both oncogene-induced senescene and replicative senescence (this upper regulation one of the most commonly used markers senescene). Melanocytic nevi (bening lesions) usually present stronger staining for p16INK4A, while melanomas, on the other hand, staining for p16INK4A is typically reduced or lost entirely (71).

Accoding to Tsao et al moderate-to-high levels of of phosphatase and tensin homologue (PTEN) expression are present in tissues like in epidermis, follicular epithelium, sebaceous and eccrine gland, and high levels of expression are present in cutaneous muscle, nerve and muscular arteries. They, also discovered that, almost all benign naevi (dysplastic nevi included) present consistenly strong PTEN levels in the cytoplasm, while a major fraction of primary cutaneous melanomas there is the absence of PTEN. This led to the conclusion that the PTEN loss has an important action in the pathogenesis of malignant melanoma (72). This is, also, supported by the finding that inactivating mutations of the PTEN tumor suppressor gene where present in 25–50% of non-familial melanomas (73).

Histologically roughly 20-50% of melanomas are associated with adjacent naevi (74), even though the rate of malignant transformation of naevi is extremely low, and most melanomas do not emerge from naevi in of high-risk individuals (those with high naevi counts) (75).

Microscopically, naevi are usually symmetric, well circumscribed and with a regular monotonous cytology. They also present two major histopathological features: nesting and maturation. Nesting is a tendency of the naevus melanocytes to group and form small clusters; maturation, is the tendency of naevi with a dermal component to gradually and progressivelly change (from superficial to deep) the nest architecture and melanocyte cytology (76).

1.3 Melanoma

Melanoma is a neoplasm that occurs from the malignant transformation of melanocytes. It is one of the most aggressive forms of skin cancer because its tendency to metastasize, presumably due to the origin of melanocytes in the neural crest (77), and the long and diffuse migration they go throught during the embrionic period. Melanocytes are found in different anatomical locations: epithelial lining of the nasal cavity, oropharynx, anus, vagina, urinary tract, skin and eye. Cutaneous melanoma represents more than 90% of melanomas, which renders early diagnosis easier (78), since most of the lesions are readily visualized without the need for complicated, expensive or invasive and risky procedures, such as internal organ biopsies of image exams.

Inspite of the intese efforts of various medical and public awareness campaigns, the incidence of cutaneous melanoma grew worldwide in the last 20 years. The risk of developing a in the United States estimated in 1 in 28 (79). According to Rige, this increase is not an

artifact owing to increased detection, but a true grow (80). This is also supported by a Danish study of 24059 melanomas diagnosed between 1985 and 2012, which showed an actual increase in incidence of melanomas of all depths (from in situ to deeply invasive), which would not be the case if the increase was only due to more frequent and earlier diagnose (81). In addition, the American Joint Committee on Cancer, found that the increased incidence involves not only all tumor thickness groups, but it is, also, not related to socio-economic status (a indicator for access to care and screening).

Among cancers, after (female) lung cancer, melanoma leads in terms of increased incidence in the Caucasian population (4). Besides the loss of life are the economic losses. Melanoma is also expensive, is the cancer with the biggest economic cost per death, as well as, the cancer causing the biggest loss of productive-years in Europe. In 2014, over 76.100 cases of invasive melanoma were diagnosed in the United States, with an estimated 9,480 deaths (82), and according to Centers for Disease Control and Prevention, and estimated cost for the US economy of US$ 3,3 billion per year (83).

Despite increases in melanoma incidence, a reduction in mortality rates among persons below the age of 65 have been observed, most likely reflecting better awareness, earlier detection and improved treatment.

In general, melanomas have a primary lateral, or horizontal, growth phase, which involves radial expansion of an in situ or superficially invasive malignant melanocyte clone, which still do not have the hability for vascular invasion or distant metastasis. In the vertical growth phase tumors demonstrate, on the histology, agglomeration of dermal cell, nests forming large nodules, the filling and expansion of the papillary dermis and growth into the reticular dermis corresponding to a flattening of the overlying epidermis (84).

Considering the facts that, lesions typically start as thin melanoma, and that the paramount element in melanoma prognosis is tumor thickness, it is easy to infer that the timing of diagnosis is of crucial importance for a better prognosis.

1.3.1 Melanoma risk factors

Fair skin (phototypes I-II), presence of multiple freckles and a lack of tanning capacity are considered risk factors for melanoma. Light hair is also a constitutional risk factor for melanoma; studies showed that individuals with light-brown, blond or red hair have at least

twice the risk of developing cutaneous melanoma in comparsion to individuals with dark-brown or black hair (85-86). According to a 2016 European consensus interdisciplinary guideline for melanoma, fair skin that burns with ease is the most common phenotypic risk factor, and the most common genotypic risk factor is inherited melanocortin-1 receptor variant (87).

On the other hand, UVR exposure (sun exposure) is the most importante environmental risk factor for melanoma. The incidence of melanoma in the UK is estimated do 10/100.000 meanwhile in Australia the estimate is 50/100.000 this, per se, is a robust indication that UVR must be paramount in its genesis. Data show that melanoma is the 4th most common tumor in Australia (considering all skin cancers togheter), while in the UK is the 18th most common tumour (88). Data analyses from diverse case-control studies show that holiday sun exposure (short intense exposure) is the main environmental risk linked with melanoma propensity, specially, in areas with temperate climates.

Sunburns also are considered a strong risk factor for melanoma, Whiteman and Green in a meta-analysis (which included 16 case–control studies regarding sunburn history) found a 2-fold increased risk of melanoma in those ever sunburned, with a 3.7-fold increase risk among those in the highest category of sunburn exposure, compared with those never sunburned (89).

Gandini et al, in a meta-analysis, consider that eye and hair color, are most likely linked to melanoma because their interaction with the indivual´s phenotype rather than being in a direct causal relation to it. (90).

Organ transplant patients, due to immunosuppression are at a significantly increased risk skin cancer (from all types, melanoma included). It was estimated that this group of individuals the risk of developing melanoma is 1.6 to 2.5 times bigger than in the general population (91).

Also, there is a two-fold increase in the risk for melanoma when a positive family history is associated; when affected first-degree relatives have multiple primary melanomas this risk is even higher. Melanoma families are also more susceptible to other types of cancers, such as, pancreatic cancer, leading to the idea that the genes involved with melanoma susceptibility may be associated in other cancer syndromes (92). Presently family history is regarded as one of the main risk factors for melanoma and there are several studies evaluating potential melanoma susceptibility genes. It is estimated that roughy 8-12% of melanoma patients have a family history.

The total count of melanocytic naevi is regarded as the most relevant marker of increased risk for melanoma (93). Some studies have even found that the total number of naevi contributes with a much higher relative risk than environmental exposure (94). According to Bataille et al., in a study with fair skin individuals from the UK, the presence of more than 100 common naevi and 2 to 3 atypical naevi gave an odds ratio between 5 and 10, whereas fair skin (types 1 and 2) was associated with a 3-fold increase in melanoma risk (95). On a cohort of teenagers diagnosed with melanoma, researchers in Australia, found that those individuals with 100 or more naevi had an odds ratio for melanoma of 46.5. The major impact of host factors in melanoma propensity was highlighted since there were no observable differences between cases and controls in relation to sun exposure habits (96). Although this data is divergent from what was stated on the previous paragraph, it emphasizes the central position of host factors in melanoma susceptibility which should lead to an increased effort on knowing those ‘‘risk phenotype’’ patients (high number of naevi) (97).

1.3.2 Melanoma diagnosis

Several studies have evince that melanomas diagnosed by physicians are in general thinner, and consequently, have a better prognosis than those that were pointed out by the patient or family members (98, 99, 100). In fact, a review encompassing 9 worldwide studies with a total 7500 patients substantiate those findings, showing that physician-detected melanomas were, in average, 0,55mm thinner than those diagnosed by the patients or their family members (101). This is also supported by an Australian study evaluating incidentally detected melanomas in which 71.9% were in situ melanomas and 28.1% were invasive melanoma, of these lesions, 94% were ‘thin’ (less than 1.0mm Breslow thickness) (102).

The standard and most widely recognized method for the screening for melanoma is full body exam (complete skin examination). This non-invasive screening examination is performed much less frequently than examinations screening for cervical and endometrial cancers, breast, prostate and colorectal in the US. Melanoma screening has not been standardized as a mainstream practice, with fewer than 25% of Americans reporting ever having received a skin examination. This is particularly surprising in the light of the Rochester Epidemiology Project, which studied 142,377 Olmsted County residents over a 4-year period and found that skin disorders were the number one reason for physician visits (103).

Almost 50% of all patients that die from melanoma are middle-aged and elderly men. In the USA only 16% of men in this subgroup report ***ever*** having been through a complete skin screening by a physician (104).

In a review from the American Academy of Dermatology skin cancer screening program, over 30 years, with more than two million skin checks during the period from 1984 to 2014, more than 20.000 melanoma cases where diagnosed. Approximately 48% of the individuals with either clinical melanoma reported that they never had their skin checked for skin cancer. Approximately three-quarters of these individuals had never been throgh a skin cancer screening before. Also, almost 80% of the patients reported not having a regular dermatologist appointment (105).

1.3.3 Melanoma subtypes

Most of the melanomas arise as "de novo" lesion, but studies showed that almost a third of the melanomas are linked with a pre-exhisting lesion. Other risk factors include chronic sun exposure (most probably intermittent and intense exposure), a propensity to burn easily and tan poorly, UVR exposure. Also associated with a increased risk for melanoma are fair complexion individuals (blond or red hair, pale skin), the presence of large congenital naevi, xeroderma pigmentosum, immunosuppression, scars, chemical exposures and Marjolin ulcers (106).

A meta-analysis found that there are two main gropus of single nucleotide polymorphisms associated with increased risk for melanoma: one associated with melanoma and total naevus count and the other with melanoma and pigmentation. The idea that there may be different (or exclusive) pathways for the development of melanoma came from the fact that these subsets of single nucleotide polymorphisms did not overlap (107).

According to the 2016 European consensus-based interdisciplinary guideline (87) for the diagnosis and treatment of melanoma, there are four classical subtypes of melanomas can be identified clinically and histologically:

> "Superficial spreading melanoma begins with an intraepidermal horizontal or radial growth phase, appearing first as a macule that slowly evolves into a plaque, often with multiple colours and pale areas of regression. Secondary nodular areas may also develop. A characteristic histologic feature is the

> presence of an epidermal lateral component with pagetoid spread of clear malignant melanocytes throughout the epidermis.
>
> Nodular melanoma in contrast is a primarily nodular, exophytic, brown-black, often eroded or bleeding tumour, which is characterised by an aggressive vertical phase, with a short or absent horizontal growth phase. Thus, an early identification in an intraepidermal stage is almost impossible. When present, an epidermal lateral component is observed histologically within three rete ridges at the maximum.
>
> Lentigo maligna melanoma arises often after many years from a lentigo maligna (melanoma in situ) located predominantly on the sun-damaged faces of older individuals. It is characterised histologically by a lentiginous proliferation of atypical melanocytes at the dermoepidermal junction and histological features of solar elastosis.
>
> Acral lentiginous melanoma is typically palmoplantar or subungual. In its initial intraepidermal phase (which may be protracted), there is irregular, poorly circumscribed pigmentation; later a nodular region reflects the invasive growth pattern. In addition, there are several rarer variants of melanoma, such as desmoplastic, amelanotic and polypoid melanomas, which constitute less than 5% of cases. Nodal melanoma in the absence of clear evidence of a primary tumour is also seen."

Whiteman et al. described two major biological subtypes of melanoma, which were classified based on clinical, histopathological, molecular, and prognostic features, and were named chronically sun-damaged skin (CSD) and non-CSD melanomas. Non-CSD is the most prevalent subtype in Caucasian people. The typical lesion is the superficial spreading melanoma that usually affects intermittently sun-exposed areas of younger individuals. Non-CSD melanoma, on molecular analysis, frequently shows the BRAFV600E mutation in approximately 70% of cases and carries a moderate mutation burden. This subtype is also more common in individuals with multiple nevi (nevus-prone phenotype) (74).

Chronic sun damage (CSD) melanomas are the second most common type of melanoma in individuals of European descent. Incidence gradually increase with older age and starts to rise around the fifth decade of life. This subtype mainly affects the face, head, neck, distal and dorsal extremities (chronically sunexposed skin), and marked solar

elastosis in the surrounding skin is typically present. Usually, they do not appear in patients with numerous melanocytic nevi, and also, typically are not associated with a precursor melanocytic nevus. Instead, affected individuals usually present with other cutaneous neoplasms associated with chronic sun exposure such as actinic keratoses, solar lentigines, or other types of non-melanoma skin cancers. All these findings combined lead to the conclusion that CSD melanomas require high cumulative doses of UV radiation to develop (74).

1.3.4 Melanoma prognosis

The paramount aspect in the prognosis of melanoma is the Breslow depth. Dr Alexander Breslow from George Washington University described in his famed work in 1970 a measurement from the granular layer to the maximum depth of melanoma invasion and noticed that the increasing depth of tumor invasion was correlated with a poorer prognosis (108).

The second most critical prognostic factor is the presence ulceration, which is defined as a combination of the following features: full-thickness epidermal defects (including absence of stratum corneum and basement membrane), evidence of host response (i.e. fibrin deposition, neutrophils), and thinning, effacement or reactive hyperplasia of the surrounding epidermis. Lastly, the mitotic rate, i.e. the number of mitoses per mm2 was added, in 2009, as an additional poor prognostic factor.

A recent study on the Journal of the American Academy of Dermatology evaluated the total number and ratio of melanoma deaths within 10 years of diagnosis for each thickness category (T1 through T4), and the risk for death did correlate with thickness from T1 to T4: 3.0% for T1, 12.4% for T2, 25.0% for T3, and 31.8% for T4, but more importantly prognosis worsened just with progression from 0.26-0.50mm through 0.76-1.00mm (109), highlighting the importance of an early diagnosis.

2. METHODS

For the present study, the data gatehered was the total number of melanocytic naevi present on the face and body of all patients that attended a private general dermatology clinic in the city of São Miguel do Oeste, Santa Catarina state, in southern Brazil.

All patients between the ages of 18 and 60 years were included in the study regardless of gender or the reason of the appointment. A total of 999 patients where enrolled between March 2016 and August 2016. All data collection and skin examinations where performed by the same dermatologist.

In 1990, the International Agency for Research on Cancer (IARC) proposed a detailed protocol to standardize methodologies in naevus epidemiological studies. It defined countable melanocytic lesions as: 'Brown to black pigmented macules or papules, which, are reasonably well defined and are darker in colour than the surrounding skin. Countable lesions do not have the features of freckles, solar lentigines, seborrhoeic keratoses, cafe-au-lait spots, or non-melanocytic lesions''. This was the standard used in this research.

Each patient had a full body examination, as is the standard practice in this clinic. The number of naevi on the face was recorded. The total number of naevi on the body was counted and standartized into three categories: 0-10, 11-50, and greater than than 50. This division was performed in accordance with similar classifications in previous studies and to facilitate data collection. To prevent misdiagnosis between naevi and ephelides, only naevi with a diameter over 2mm were included, even lesions greater than 6mm where also accouted as melanocytic nevi, no distinction made on atypical nevi, dermal nevus (papulous lesions) where also accounted.

Data regarding patients' age, skin type, body mass index and eye color where, also, collected in accordance with previous studies (110-111). The skin phototype classification was performed according to Fitzpatrick's scale of I to VI as follows: Fitzpatrick I always burns, never tans; II usually burns, tans less than average with difficulty; III sometimes burns mildly, tans about average; IV rarely burns, tans more than average with ease; V never burns, always tans, brown skin; VI never burns, black skin (112).

The eye color classification was also performed in accordance with previous studies. Patients with blue and green eye color were classified as having a light eye color, whereas those with brown and black eye color were classified as as having a dark eye color (113).

In Brazil hair dyeing is extremely common and asking patients about their natural hair color could potentially generate unreliable data. As such, hair color was not documented in this study.

Data regarding body mass index (BMI) was also collected, since there are some studies affirming that there may be a correlation between BMI and the number of naevi in the body (114-115). Also, some evidence suggests that the tanning ability and skin complexion (pigmentation of hair, skin and eyes) are regulated by genes that may be associated with intrinsic factors that produce obesity (116). Furthermore, a recent study found obesity to be an indepdent clinical risk factor for thick primary cutaneous melanomas (117).

Besides that, other important risk factors are intense sun exposure during childhood, and history of sunburns, but the definition of sunburns varies across the literature, and most individuals can not remember precisely the quantity of painful sunburns in childhood, for this reason, this was not accounted in the presente study.

There was no need for specific informed consent, since all the patients attending the clinic routinely undergo a full body examination (unless refused by the patient). Furthermore, there was no therapeutic intervention, photographic documentation, or identification based on personally identifiable data. Indeed, other than the number of naevi on the face and body, the remaining data (eye color, BMI, skin phototype, etc) are routinely recorded. Finally, to overcome the risk of accouting a patient twice (for example in a follow up visit) each patient that was included has in his prontuary a note saying the he had already been accounted on the study.

For the statistic analyze, quantitative data where described by amplitude, and categorical information where presented by counting and percentuals.

In order to evaluate the correlation between the number of nevi on the face and on the body, the Spearman correlation coefficient was utilized. To estimate the predictive probability of the number of nevi on the face for the number of total nevi on the body the receiver operating characteristics curve (ROC) was utilized. A logistic regression model was elaborated to estimate the probability of more than 50 nevi on the body being present according to the number of nevi on the face. P values under 0,05 where considered statistically significant. Data where analyzed through the SPSS program, version 22.0.

3. RESULTS

Females comprised the majority of cohort in this study, with 614 (61,4%) individuals, and 385 (38,5%) males. The age group from 18-30 years was the most common one with 328 (32,8%) patients. The smallest group was aged between of 31-40 with 197 (19,7%) individuals. The group from 41 to 50 years had a total of 227 (22,7%) persons, and the group from 51 to 60 years 247 (24,7%) persons (being the second group in number of individuals).

The eye color was evenly distributed with 499 (50%) persons with light eyes and 500 (50%) persons with dark eyes.

Regarding to the skin type 418 (41,8%) persons had Fitzpatrick II (the most common phototype) closely followed by 401 (40%) persons with phototype III. The lighter persons were the third most common group, with 131 (13%) individuals with phototype I. Darker individuals where a minority with 46 (4,5%) having type IV skin, and only 3 (0,3%) with type V, the darker patients represented only 5% of the total.

Body Mass Index (BMI) had 27 (2,7%) individuals with a BMI below 18, 606 (60,6%) individuals from 19-25, representing the gross majority of the cohort. Twenty six percent of the individuals had a BMI of 26-30 (259 persons), and 102 (10%) persons between 31-45.

From the total of 999 individuals, 316 (%) persons had between 0-10 naevi on the body; 429 (%) had between 11-50 naevi (being the most numerous group), and 254 (%) inviduals had more than 50 naevi on the body, which means that approximately a quarter of the cohort in this sample had more than 50 total naevi on total body count (Figure 1).

One hundred and fifthy one persons (15%) had no naevi on the face, and 667 (66,7%) persons had between 0 and 4 facial naevi. A group of 240 (24%) individuals had between 5-10 naevi on the face. Ninety-two (9,2%) persons had a facial nevus count varying between 11-23 naevi; 23 was the maximum total count on the face seen in 2 (0,2%) persons. A total of 90.8% persons had between 0-10 naevi on the face.

Thirtheen individuals had zero naevi on the face and more then 50 naevi on the body this represents roughly 1,3% of all the cohort and from this subgroup six (0,6%) individuals had a skin phototype I, four (0,4%) persons phototype II and three (0,3%) persons phototype III.

There was no difference with respect to the number of naevi in correlation with eye color. Also, it hasn't been found any statistical correlation between body mass index and the number of naevi.

The data collected revealed a strong correlation between the total number of naevi on the body and the number of naevi on the face with a Spearman coefficient of 0,62. ($rS=0,62$; $p<0,001$). Additionally, in a logistic regression analysis, it was shown that the significant relation of naevi in the face is independent of the other factors, since it remains significant in the presence of those in the model.

The cohort was divided into two groups based on the total number of naevi on the body, from 0-50 naevi and more than 50 naevi and the data was posted on a ROC curve with a very good result of 0,838 (Figure 2).

On average individuals who had between 0-<10 nevi on the body had an average of 1,5 nevi on the face. Individuals who had between 11-50 nevi on the body, had an average of 3,7 nevi on the face; and finally, persosns with more than 50 nevi on the body had an average of 8,24 nevi on the face (Table 1).

3.1 Estimates

From the total of 999 subjects, 265 (25,6%) had 6 or more naevi on the face. If 6 naevi is utilized as a cutting point (for which individuals need a full body examination) approximately 27% of the cohort would need a complete skin check (roughly one out four individuals), with a probability of detection of 69% (sensitivity).

If 5 naevi on the face becomes the cutting point, it would be necessary to exam 33% (only a third) of the total, with a sensitivity of 74%.

Likewise, with 4 naevi on the face as the cutting point it would be required to exam 44% (almost one out of each two) of the sample, and this would give a sensitivity of 81%.

4. DISCUSSION

Ultraviolet rays in sunlight are the main environmental determinant of melanoma, whereas the number of melanocytic naevi is the main host risk indicator.

Melanoma, inspite of being an aggressive tumor, has an advantage over other tumors since most of the lesions are readily available to naked eye examination. Skin tumors are easy to screen for, and the screening is painless, and relatively cheap (comparing to other types of cancers screening) without the need of expensive equipment.

Ia a study 80% of the melanomas detected by the dermatologist were casual diagnosis during routine examinations for distinct complaints, this same study also showed that melanomas detected by non-dermatologists health care professionals are in general thinner than those self detected by patients, but, primary care practitioners encounter many pitfalls and difficulties in the screening of skin cancer that may contribute to treatment delay (118). Pennie et al. (2007) evaluated Breslow thickness on 2020 patients with diagnosis made on a dermatologist and nondermatologist, at 6 months the dermatologist detection group had a survival rate of 98% the nondermatologist group 95%. At 2 years the rates were 87% (dermatologist) versus 79% (nondermatologist), and at 5 years 74% against 69%. This data reflects that there is a marked contrast in melanoma stage (at the diagnosis) by provider type, with a prevalence of early-stage melanoma (stage 0, or stage I or II) in the dermatologist group and of late-stage melanoma (stage III or stage IV) in the nondermatologist group (119).

Several studies report comparable results with dermatologists constantly diagnosing thinner melanomas than dermatologist staff (nurse practitioners and/or physician assistant), patients themselves, and also, primary care providers. This is important to emphasize because the number of dermatologist detected melanomas may not seem so divergent than patient diagnosed melanomas, but from a prognostic point of view this makes a huge difference. This is corroborated by the study from the Veterans Affair in Minneapolis on the invasive incidental melanomas, almost 89% of these had a Breslow depth of less than 1mm compared with 58% of the invasive consult melanomas (71).

In the far year on 1992 it was calculated that 63% of patients who had a melanoma diagnosis where seen by their general practitioner in the year before diagnosis; and only 20% of these individuals had been submited to a full skin examination (120). More recent evidence from the year 2000 and also from 2010 National Health Interview Survey (an annual survey of US adults), showed significant increases in overall national total body skin examination (TBSE) screening rates between 2000 and 2010. In 2000, 7.6% of the population reported

undergoing a TBSE within the previous year; in 2010, the number grew to 10.6% (121). According to the American Academy of Dermatology only 30% of the dermatologists screen all patients for skin cancer (inspite of the cause of the visit), and most state lack of time to perform as the main reason to not completely examinate all the skin (122). The author can say by his personal experience (from visiting different dermatology clinics and hospitals, in Brazil, Argentina, Colombia, United States and Austria, that only a small amount of the dermatologists do exam all patients thoroughly.

Additionally, a survey showed that the number of dermatologists remain stable in the US in the last three decades, and also, that more than 30% dermatologists are seeking to hire additional physician for their practice, showing that there is an unmet demand. This same survey demonstrated that the overall work hours in the dermatology practices did not change, but dermatologists have spent more time in surgical and probably cosmetic dermatology, leading into a decline in the time spent in the care of patients with medical dermatologic conditions (123). In this same topic several, dermatology practices are hiring nonphysician clinicians, the number of assoiciateds in the The Society for Dermatology Physician Assistants grew exponentially from 15 in 1994 to more than 1200 in 2006. Also, those dermatologists who spend half or more of their patient care time on surgical and cosmetic dermatology were more prone, than others, to use physician's assistants or nurse practitioner, professionals without the same level of training for the early diagnose of melanoma (124).

Studies showed that a complete skin examination (considering only the time spent to actually exam the patient, excluding interviewing and undressing and dressing) may take from 1,1minutes without dermoscopy, and 2,4 minutes with dermoscopy (125), this difference, that in a quick look may seem huge, becomes smaller when taking in consideration that the average dermatological visit in the US is 15,3 minutes (based on 29554 records from 1993 to 2010) (126). According to a German report the average dermatologic consultation is 23 minutes (127). Also, from Germany, a study with 347 patients with melanoma diagnosis showed that in 66.9% a examination of the naevi with dermatoscope was executed during the consultation that gave rise to the wariness or to the diagnosis of melanoma. This same study showed that patients enrolled in specialized dermoscopic screening programs presented the smallest mean Breslow thickness (0.49mm) (128), which emphasizes the need of surveillance.

An Irish study, from Aldridge et al. (2013), with 1851 patients focused on full body skin examination of patients which already have been refered to a specialized dermatologic clinic (for what was called a "index" lesion) showed substantial proportion of incidental melanomas

detected because TBSE was performed. This single index lesion approach produced a rate of melanoma diagnosis of 1.3% (24/1851) in comparsion to 2.1% (38/1,851) for the evaluation with a total body skin examination, implying that not performing a TBSE would result in over one third of the melanomas being missed (129).

A study from California found a reduction on thick melanoma rates, when patients with a personal melanoma history or a family history, and more than 50 naevi where followed with full body examination, full body photography and dermoscopy (130). This finding also corroborates another study in which patients with more than 20 naevi had thinner melanomas as result of closer follow up (131). This is, additionally, ratified by data demonstrating that, the admission in a routine schedule for dermoscopic evaluation and follow-up was the most critical aspect correlatd (both in uni and multivariate analysis) with thinner melanomas.

Still validating the need of "knowing" the patient's skin Schwartz et al., analyzed individual characteristics associated with thinner melanomas and found that individuals that had more than 20 clinically benign naevi, a personal history of melanoma or at least 1 atypical naevus had significantly thinner melanomas.

Evaluation on the data from the National Program for Cancer Registries; National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER); and the US Centers for Disease Control and Prevention's National Vital Statistics System, comparing mortality rates from melanoma and dermatologist density found that the presence of a single dermatologist for each group of 100.000 people reduced melanoma mortality by 35%, compared to those counties with none dermatologists (132).

In the other hand, Moran et al. (2011), evaluating 483 in a nine a month period, found only three patients (0,6% of the total) with melanomas (133). Also, in a private office retrospective chart review of the records from 50,699 patients that had undergone complete full skin examination during a 10 years period, only 235 cases of melanoma where diagnosed, representing only 0,46% of the total sample (134), this is very similar to a Veterans Affair study in Minneapolis that found 0,5% of incidental melanomas in a group of 17,174 patients. Still corroborating these data two different studies, one stating that about 2.000 patients (aged between 14 and 34 years) had to evaluated for each diagnosis melanoma (135); and another in Schleswig-Holstein stating that 620 needed to be evaluated for each diagnosis of melanoma (in the age group of 20 years and older)(136).

A Belgian study with 1982 patients comparing total skin examination and "lesion direct examination" of lesions pointed out by the patients found similar detection rates (considering

melanoma and non-melanoma) 2,3% and 3,2% respectivelly and this was not statistically significant. But the amount of time spent with total skin examination (accouting the time for the patient to undress) was 5.6 times longer than with lesion direct exam. The total cost for every diagnose of skin cancer was calculated in €931 (US $1008) for the lesion direct examination and €1012 (US $1096) for total skin examination (137).

Regarding to harmness of the clinical visual skin examination screening, in the German SCREEN study, approximately 4.4% of screened individuals (1 of 23 participants) had to go through to an excision for a dubious lesion, and most of these biopsies did not reveal cancer (not only melanoma). Also, in general for both genders, 28 excisions had to be performed for every diagnose of melanoma. It was calculated that this translated into one less death caused by melanoma for every 100 000 persons evaluated (138). Even this drop in mortality between 2007 and 2010 is questioned and is theorized to be due to a reduction in out-of-hospital skin melanoma deaths. The authors of this study speculate that death-certifying private physicians in the federal state of Schleswig-Holstein who strongly believed in the mortality benefit of the skin cancer screening were hesitant to certify skin melanoma deaths among enrolled in the SCREEN project (139).

Addicionaly there is the risk of overdiagnosis and overtreatment (the diagnosis and treatment of a lesion that would otherways never have posed any harm for the individual in the lack of screening and treatment) as additional major potential risks of widespread screening (140).

Despite of all the data regarding the growing occurence of melanoma and the paramount importance of early diagnosis on the prognosis, still, studies were not able to confirm that skin cancer screening of the general population (by means of total-body examination) in asymptomatic persons is cost-effective (141); and also supporting theses findings the United States Preventive Services Task Force (USPSTF) concludes that ”the current evidence is insufficient and that the balance of benefit and harms of visual skin examination by a clinician to screen for skin cancer in asymptomatic adults (adults who do not have a history of premalignant or malignant skin lesions) cannot be determined” but recognize that screening in high-risk populations may be important.

It is very important to consider, inspite of all the aforementioned data, that there is still no other method, so fast, easily achievable, and cheap for the early diagnosis of melanoma rather than physical examination (still no CT scans, no PET scans or laboratory tests, and it is important to reiterate the need for and early diagnosis as was previously cited by a recent

study from Landow et al, which calculated the proportion of melanoma deaths within 10 years of diagnosis for each thickness category T1 to T4: 3.0% for T1, 12.4% for T2, 25.0% for T3, and 31.8% for T4, but more importantly prognosis worsened just with progression from 0.26-0.50 mm through 0.76-1.00mm.

The need for a fast and reliable tool to predict which patients are in risk for melanoma is very important, specially, for general practitioners. According to a study from England and Whales skin conditions are the number one complain that makes people look for a general practitioner, with an estimate of 24% of whole population seeking attention because skin related conditions (142). Also, in accordance with a previously mentioned study, the total cost of a general skin cancer screening for patients of ages between 14-35 years was €433 756 and resulted in the detection of only 6 melanomas. The cost of treatment for patients with metastatic melanoma was estimated in US$35,472 (per patient per month) for Ipilimumab and US$17,793 (per patient per month) for the vemurafenib cohort, including medication, office visits, etc, older medications like Dacarbazine had an estimated cost about $15,883 (per patient per month) (143).

Since the total naevi count is an major indicator of melanoma risk, and a full body examination with dermoscopy consumes less than 20% of the total time of the average dermatologic appointment, it's easy to presume that with simple full skin examination of the patients the goal of diagnosing melanomas at a early stage could be achieved; but reality is different from theory.

Our results are in accordance with a German study that found approximately 51% of the patients had between 11-50 melanocytic naevi (being the most numerous group), our data showed 42% of the patients on the group between 11-50 melanocytic naevi, being also the most numerous group.

The number of melanocytic naevi in the arm has been postulated as good predictor of the total number of naevi, using 5 naevi as a cut-off point the sensivity was 94% and the specificity 71% for diagnosing those patients with more than 50 melanocytic naevi in the body (144). Although it seems practical having the arms examinated requires the patient to partially undress, specially in cold weather climates this may take some minutes, hence the need for an easier marker of patients in risk of having 50 or more melanocytic naevi.

Argenziano et al. (2012) in a study with more than 14.000 patients that went through complete skin examination (patients that would not, normally, receive a complete skin check, since they were looking for a dermatologist because of a focused skin complaint, located on a

small body area) found that approximately 400 patients had to be examined to diagnose one melanoma (145). If this data is crossed with data from the present study the screening based on the number of naevi on the face could reduce considerably the number need to examinate. If 5 naevi on the face is utilized as a cutting point (with a sensitivity of approximately 75%), about 33% of the patients would require a complete skin check. Also, if we use 4 naevi on the face as the cutting point we would be required to exam 44% (almost one out of each two) of the sample, and this would give a sensitivity of 81%. It is important to highlight that this two studies are comparing different aspects; the first focused on melanoma detection, and the second on the risk factor of nevus count on the body (which is the main phenotypical risk for melanoma).

In comparsion with data from the study of the twenty naevi on the arms where the sensitivity was of 65,5% for the patients younger than 50 years and only 37,5% for the patients above 50 years of age, the results in the present research showed a overall sensitivity of 81% for the risk of having more than 50 total naevi on the body if the patient has more than 4 naevi on the face (considering all ages groups and different skin types and both sexes) (146).

The results of this research bring surprisingly similar results with the 20 arm nevi study from Argenziano et al. (2014) (in terms of sensitivity, specificity, false-negative and false-positive) when 8 nevi are used as a cutting point. Below the comparsion between the two studies (Table 2 and Table 4).

On the Spearman correlation if seven nevi on the face are used as reference sensitivity will be 64,2% with a specificity of 92,1%.

Also, in this research the logistic regression analysis show that the significative relation between the number or nevi on the face with the possibility of having more than 50 nevi on the body is independent of other factors, since it stays significative even the presence of those in the model.

Finally, a simple, fats, low cost and readly avaliable tool to undercover those patients with more than 50 naevi on the body, can be usefull for doctors of all medical fields, specially it this information starts being taught on medical schools, this can become a widespread information firstly among doctors, and later on among the general population.

5. REFERENCES

1. Hanly P, Soerjomataram I, Sharp L. Measuring the societal burden of cancer: The cost of lost productivity due to premature cancer-related mortality in Europe. Int J Cancer 2015 Feb;136(4):E136-45.

2. U.S. Department of Health & Human Services. 2019 [access in 2019 aug 05]. Available in: www.cdc.gov/nchs.

3. Sober AJ. Cutaneous melanoma: opportunity for cure. CA Cancer J Clin 1991 Jul-Aug;41(4):197-9.

4. Little EG, Eide MJ. Update on the current state of melanoma incidence. Dermatol Clin 2012 Jul; 30(3):355-61.

5. de Vries E, Coebergh JW. Melanoma incidence has risen in Europe. BMJ 2005 Sep 24;331(7518):698.

6. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA. Increasing Burden of Melanoma in the United States. J Invest Dermatol 2009 Jul;129(7):1666-74.

7. Rigel DS, Friedman RJ, Kopf AW. The incidence of malignant melanoma in the United States: issues as we approach the 21st century. J Am Acad Dermatol 1996 May;34(5 Pt 1):839-47.

8. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. CA Cancer J Clin 2006 Mar-Apr;56(2):106-30.

9. Marks R. An overview of skin cancers: incidence and causation. Cancer 1995 Jan 15;75(2 Suppl):607-12.

10. Vecchiato A, Zonta E, Campana L, Dal Bello G, Rastrelli M, Rossi CR, et al. Long-term Survival of Patients With Invasive Ultra-thin Cutaneous Melanoma: A Single-center Retrospective Analysis. Medicine (Baltimore) 2016 Jan;95(2):e2452.

11. Winston W Tan. Malignant Melanoma. 2016 [acesso em 02 jul 2019]; Disponível em: https://emedicine.medscape.com/article/280245-overview.

12. Ribero S, Glass D, Bataille V. Genetic epidemiology of melanoma. Eur J Dermatol 2016 Aug 1;26(4):335-9.

13. Haenssle HA, Mograby N, Ngassa A, Buhl T, Emmert S, Schön MP, et al. Association of Patient Risk Factors and Frequency of Nevus-Associated Cutaneous Melanomas. JAMA Dermatol 2016 Mar;152(3):291-8.

14. Ribero S, Zugna D, Osella-Abate S, Glass D, Nathan P, Spector T, et al. Prediction of high naevus count in a healthy U.K. population to estimate melanoma risk. Br J Dermatol 2016 Feb;174(2):312-8.

15. Bauer J, Garbe C. Acquired melanocytic nevi as risk factor for melanoma development: a comprehensive review of epidemiological data. Pigment Cell Res 2003 Jun;16(3):297-306.

16. Bataille V, Bishop JA, Sasieni P, Swerdlow AJ, Pinney E, Griffiths K, et al. Risk of cutaneous melanoma in relation to the numbers, types and sites of naevi: a case-control study. Br J Cancer 1996 Jun;73(12):1605-11.

17. Smith RA, Cokkinides V, Brawley OW. Cancer screening in the United States, 2009: a review of current American Cancer Society guidelines and issues in cancer screening. CA Cancer J Clin 2009 Jan-Feb;59(1):27-41.

18. Siskind V, Hughes MC, Palmer JM, Symmons JM, Aitken JF, Martin NG. Nevi, family history, and fair skin increase the risk of second primary melanoma. J Invest Dermatol 2011 Feb;131(2):461-7.

19. Shaikh WR, Dusza SW, Weinstock MA, Oliveria SA, Geller AC, Halpern AC. Melanoma Thickness and Survival Trends in the United States, 1989 to 2009. J Natl Cancer Inst 2015 Nov 12;108(1). pii: djv294.

20. Margolis DJ, Halpern AC, Rebbeck T, Schuchter L, Barnhill RL, Fine J, et al. Validation of a melanoma prognostic model. Arch Dermatol 1998 Dec;134(12):1597-601.

21. Federman DG, Kravetz JD, Kirsner RS. Skin cancer screening by dermatologists: prevalence and barriers. J Am Acad Dermatol 2002 May;46(5):710-4.

22. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005 Jan;41(1):28-44.

23. McGuire ST, Secrest AM, Andrulonis R, Ferris LK. Surveillance of patients for early detection of melanoma: patterns in dermatologist vs patient discovery. Arch Dermatol 2011 Jun;147(6):673-8.

24. Gilbert SF. Developmental Biology. 6th ed. Sunderland: Sinauer Associates, 2000.

25. Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. Eur J Dermatol 2002 Jul-Aug;12(4):390-9.

26. Dasgupta A, Katdare M. Ultraviolet Radiation-Induced Cytogenetic Damage in White, Hispanic and Black Skin Melanocytes: A Risk for Cutaneous Melanoma. Cancers (Basel) 2015 Aug 14;7(3):1586-604.

27. Nasti TH, Timares L. MC1R, eumelanin and pheomelanin: their role in determining the susceptibility to skin cancer. Photochem Photobiol 2015 Jan-Feb;91(1):188-200.

28. Zaidi MR, De Fabo EC, Noonan FP, Merlino G. Shedding light on melanocyte pathobiology in vivo. Cancer Res 2012 Apr 1;72(7):1591-5.

29. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. Photochem Photobiol 2008 May-Jun;84(3):539-49.

30. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new. J Eur Acad Dermatol Venereo 2003 Nov;17(6):663-9.

31. Abdel-Malek ZA, Ruwe A, Kavanagh-Starner R, Kadekaro AL, Swope V, Haskell-Luevano, et al. alpha-MSH tripeptide analogs activate the melanocortin 1 receptor and reduce UV-induced DNA damage in human melanocytes. Pigment Cell Melanoma Res 2009 Oct;22(5):635-44.

32. von Koschembahr AM, Swope VB, Starner RJ, Abdel-Malek ZA. Endothelin-1 protects human melanocytes from UV-induced DNA damage by activating JNK and p38 signalling pathways. Exp Dermatol 2015 Apr;24(4):269-74.

33. Mitra D, Luo X, Morgan A, Wang J, Hoang MP, Lo J, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature 2012 Nov 15;491(7424):449-53.

34. Morgan AM, Lo J, Fisher DE. How does pheomelanin synthesis contribute to melanomagenesis?: Two distinct mechanisms could explain the carcinogenicity of pheomelanin synthesis. BioEssays 2013 Aug;35(8):672-6.

35. Grichnik JM, Ross AL, Schneider SL, Sanchez MI, Eller MS, Hatzistergos KE. How, and from which cell sources, do naevi really develop? Exp Dermatol 2014 May;23(5):310-3.

36. McCalmont T. Melanocytic Naevi. Medscape. 2016 [acesso em 02 jul 2019]; Disponível em: http://emedicine.medscape.com/article/1058445-overview#a6.

37. Huang JM, Chikeka I, Hornyak TJ. Melanocytic Nevi and the Genetic and Epigenetic Control of Oncogene-Induced Senescence. Dermatol Clin 2017 Jan;35(1):85-93.

38. Levy R, Lara-Corrales I. Melanocytic Nevi in Children: A Review. Pediatr Ann 2016 Aug 1;45(8):e293-8.

39. de Maleissye MF, Beauchet A, Saiag P, Corrêa M, Godin-Beeckmann S, Haeffelin M, et al. Sunscreen use and melanocytic nevi in children: a systematic review. Pediatr Dermatol 2013 Jan-Feb;30(1):51-9.

40. Newton-Bishop JA, Chang YM, Iles MM, Taylor JC, Bakker B, Chan M, et al. Melanocytic nevi, nevus genes, and melanoma risk in a large case-control study in the United Kingdom. Cancer Epidemiol Biomarkers Prev. 2010 Aug;19(8):2043-54.

41. Clark WH Jr, Reimer RR, Greene M, Ainsworth AM, Mastrangelo MJ. Origin of familial malignant melanomas from heritable melanocytic lesions. 'The B-K mole syndrome'. Arch Dermatol 1978 May;114(5):732-8.

42. Clark WH Jr, Elder DE, Guerry D, Epstein MN, Greene MH, Van Horn M. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. Hum Pathol 1984 Dec;15(12):1147-65.

43. Schäfer T, Merkl J, Klemm E, Wichmann HE, Ring J, KORA Study Group. The epidemiology of nevi and signs of skin aging in the adult general population: Results of the KORA-survey 2000. J Invest Dermatol 2006 Jul;126(7):1490-6.
44. Tsao H, Sober AJ. Atypical melanocytic nevi. Freedburg IM, ed. Fitzpatrick's Dermatology in General Medicine. 6th ed. New York, NY: McGraw-Hill; 2003. p. 906-16.

45. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005;41(1):28-44.

46. Schaffer JV. Update on melanocytic nevi in children. Clin Dermatol 2015 May-Jun;33(3):368-86.

47. Gallagher RP, Rivers JK, Lee TK, Bajdik CD, McLean DI, Coldman AJ. Broad-spectrum sunscreen use and the development of new nevi in white children: A randomized controlled trial. JAMA 2000 Jun 14;283(22):2955-60.

48. Rodvall Y, Wahlgren CF, Ullén H, Wiklund K. Common melanocytic nevi in 7-year-old schoolchildren residing at different latitudes in Sweden. Cancer Epidemiol Biomarkers Prev 2007 Jan;16(1):122-7.

49. Pack GT, Lenson N, Gerber DM. Regional distribution of moles and melanomas. AMA Arch Surg 1952 Dec;65(6):862-70.

50. Nicholls EM. Development and elimination of pigmented moles, and the anatomical distribution of primary malignant melanoma. Cancer 1973 Jul;32(1):191-5.

51. Karlsson MA, Rodvall Y, Wahlgren CF, Wiklund K, Lindelöf B. Similar anatomical distributions of childhood naevi and cutaneous melanoma in young adults residing in northern and southern Sweden. Eur J Cancer 2015 Sep;51(14):2067-75.

52. Naldi L, Lorenzo Imberti G, Parazzini F, Gallus S, LaVecchia C. Pigmentary traits, modalities of sun reaction, history of sunburns, and melanocytic nevi as risk factors for cutaneous malignant melanoma in the Italian population - Results of a collaborative case-control study. Cancer 2000 Jun 15;88(12):2703-10.

53. Siskind V, Darlington S, Green L, Green A. Evolution of melanocytic nevi on the faces and necks of adolescents: a 4 y longitudinal study. J Invest Dermatol 2002 Mar;118(3):500-4.

54. Scope A, Marchetti MA, Marghoob AA, Dusza SW, Geller AC, Satagopan JM. The study of nevi in children: Principles learned and implications for melanoma diagnosis. J Am Acad Dermatol 2016 Oct;75(4):813-23.

55. English DR, Armstrong BK. Melanocytic nevi in children. I. Anatomic sites and demographic and host factors. Am J Epidemiol 1994 Feb 15;139(4):390-401.

56. Kittler H, Pehamberger H, Wolff K, Binder M. Follow-up of melanocytic skin lesions with digital epiluminescence microscopy: patterns of modifications observed in early melanoma, atypical nevi, and common nevi. J Am Acad Dermatol 2000 Sep;43(3):467-76.

57. Lin WM, Luo S, Muzikansky A, Lobo AZ, Tanabe KK, Sober AJ, et al. Outcome of patients with de novo versus nevus-associated melanoma. J Am Acad Dermatol 2015 Jan;72(1):54-8.

58. Tsao H, Bevona C, Goggins W, Quinn T. The transformation rate of moles (melanocytic nevi) into cutaneous melanoma: a population-based estimate. Arch Dermatol 2003 Mar;139(3):282-8.

59. Zalaudek I, Hofmann-Wellenhof R, Kittler H, Argenziano G, Ferrara G, Petrillo L, et al. A dual concept of nevogenesis: theoretical considerations based on dermoscopic features of melanocytic nevi. J Dtsch Dermatol Ges 2007 Nov;5(11):985-92.

60. Zalaudek I, Marghoob AA, Scope A, Hofmann-Wellenhof R, Ferrara G, Argenziano G. Age distribution of biopsied junctional naevi - Unna's concept versus a dual concept of nevogenesis. J Am Acad Dermatol 2007 Dec;57(6):1096-7.

61. Fortina AB, Zattra E, Bernardini B, Alaibac M, Peserico A. Dermoscopic Changes in Melanocytic Naevi in Children during Digital Follow-up. Acta Derm Venereol 2012 Jul;92(4):427-9.

62. Scope A, Dusza SW, Marghoob AA, Satagopan JM, Braga Casagrande Tavoloni J, Psaty EL, et al. Clinical and dermoscopic stability and volatility of melanocytic nevi in a population-based cohort of children in framingham school system. J Invest Dermatol 2011 Aug;131(8):1615-21.

63. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 2003 Jun 4;95(11):806-12.

64. Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, et al. High frequency of BRAF mutations in nevi. Nat Genet 2003 Jan;33(1):19-20.

65. Ross AL, Sanchez MI, Grichnik JM. Molecular Nevogenesis. Dermatol Res Pract 2011;2011:463184.

66. Moreno S, Soria X, Martínez M, Martí RM, Casanova JM. Epidemiology of Melanocytic Naevi in Children from Lleida, Catalonia, Spain: Protective Role of Sunscreen in the Development of Acquired Moles. Acta Derm Venereol 2016 May;96(4):479-84.

67. Poynter JN, Elder JT, Fullen DR, Nair RP, Soengas MS, Johnson TM, et al. BRAF and NRAS mutations in melanoma and melanocytic nevi. Melanoma Res 2006 Aug;16(4):267-73.

68. Hui P, Perkins A, Glusac E. Assessment of clonality in melanocytic nevi. J Cutan Pathol 2001 Mar;28(3):140-4.

69. Kittler H, Seltenheim M, Dawid M, Pehamberger H, Wolff K, Binder M. Frequency and characteristics of enlarging common melanocytic nevi. Arch Dermatol 2000 Mar;136(3):316-20.

70. Damsky W, Micevic G, Meeth K, Muthusamy V, Curley DP, Santhanakrishnan M, et al. mTORC1 activation blocks BrafV600E-induced growth arrest but is insufficient for melanoma formation. Cancer Cell 2015 Jan 12;27(1):41-56.

71. Funk JO, Schiller PI, Barrett MT, Wong DJ, Kind P, Sander CA. p16INK4a expression is frequently decreased and associated with 9p21 loss of heterozygosity in sporadic melanoma. J Cutan Pathol 1998 Jul;25(6):291-6.

72. Tsao H, Mihm MC Jr, Sheehan C. PTEN expression in normal skin, acquired melanocytic naevi, and cutaneous melanoma. J Am Acad Dermatol 2003 Nov;49(5):865-72.

73. Wu H, Goel V, Haluska FG. PTEN signaling pathways in melanoma. Oncogene May 19;22(20):3113-22.

74. Whiteman DC, Pavan WJ, Bastian BC. The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. Pigment Cell Melanoma Res 2011 Oct;24(5):879-97.

75. Kraft S, Tsao H. Melanoma-associated naevi: precursors or coincidence? Br J Dermatol 2015 Sep;173(3):633-4.

76. Ferringer T. Update on immunohistochemistry in melanocytic lesions. Dermatol Clin 2012 Oct;30(4):567-79.

77. Hanly P, Soerjomataram I, Sharp L. Measuring the societal burden of cancer: the cost of lost productivity due to premature cancer-related mortality in Europe. Int J Cancer 2015 Feb 15;136(4):E136-45.

78. Ferreira FR, Nascimento LF. Mortality due to cutaneous melanoma in south region of Brazil: a spatial approach. An Bras Dermatol 2016 Jul-Aug;91(4):437-41.

79. Glazer AM, Winkelmann RR, Farberg AS, Rigel DS. Analysis of Trends in US Melanoma Incidence and Mortality. JAMA Dermatol 2017 Feb 1;153(2):225-6.

80. Rige DS. Trends in dermatology: melanoma incidence. Arch Dermatol 2010 Mar;146(3):318.

81. Helvind NM, Hölmich LR, Smith S, Glud M, Andersen KK, Dalton SO, et al. Incidence of In Situ and Invasive Melanoma in Denmark From 1985 Through 2012: A National Database Study of 24?059 Melanoma Cases. JAMA Dermatol 2015 Oct;151(10):1087-95.

82. Siegel RL, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29.

83. U.S. Department of Health & Human Services. Surgeon General Call to Action to Prevent Skin Cancer: Exec Summary. 2011 [access in 2019 jul 11]. Available in: https://www.hhs.gov/surgeongeneral/reports-and-publications/skin-cancer/executive-summary/index.html.

84. Wick MR. Cutaneous melanoma: A current overview. Semin Diagn Pathol 2016 Jul;33(4):225-41.

85. Grulich AE, Bataille V, Swerdlow AJ, Newton-Bishop JA, Cuzick J, Hersey P, et al. Naevi and pigmentary characteristics as risk factors for melanoma in a high-risk population: a case-control study in New South Wales, Australia. Int J Cancer 1996 Aug 7;67(4):485-91.

86. Ródenas JM, Delgado-Rodriguez M, Herranz MT, Tercedor J, Serrano S. Sun exposure, pigmentary traits, and risk of cutaneous malignant melanoma: a case-control study in a Mediterranean population. Cancer Cause Control 1996 Mar;7(2):275-83.

87. Garbe C, Peris K, Hauschild A, Saiag P, Middleton M, Bastholt L. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline - Update 2016. Eur J Cancer 2016 Aug;63:201-17.

88. Armstrong BK, Kricker A. Cutaneous melanoma. Cancer Survey 1994;19(20):219-40.

89. Whiteman D, Green A. Melanoma and sunburn. Cancer Causes Control 1994 Nov;5(6):564-72.

90. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer 2005 Sep;41(14):2040-59.

91. Dinh QQ, Chong AH. Melanoma in organ transplant recipients: the old enemy finds a new battleground. Australas J Dermatol 2007 Nov;48(4):199-207.

92. Hawkes JE, Truong A, Meyer LJ. Genetic predisposition to melanoma. Semin Oncol 2016 Oct;43(5):591-97.

93. Smith RA, Cokkinides V, Brawley OW. Cancer screening in the United States, 2009: a review of current American Cancer Society guidelines and issues in cancer screening. CA Cancer J Clin 2009 Jan-Feb;59(1):27-41.

94. Garbe C, Buttner P, Weiss J, Soyer HP, Stocker U, Kruger S, et al. Associated factors in the prevalence of more than 50 common melanocytic naevi, atypical melanocytic naevi, and actinic lentigines: multicenter case–control study of the Central Malignant

Melanoma Registry of the German Dermatological Society. J Invest Dermatol 1994 May;102(5):700-5.

95. Bataille V, Bishop JA, Sasieni P, Swerdlow AJ, Pinney E, Griffiths K, et al. Risk of cutaneous melanoma in relation to the numbers, types and sites of naevi: a case–control study. Br J Cancer 1996 Jun;73(12):1605-11.

96. Youl P, Aitken J, Hayward N, Hogg D, Liu L, Lassam N, et al. Melanoma in adolescents: a case-control study of risk factors in Queensland, Australia. Int J Cancer 2002 Mar 1;98(1):92-8.

97. Bataille V, Grulich A, Sasieni P, Swerdlow A, Newton Bishop J, McCarthy W, et al. The association between naevi and melanoma in populations with different sun exposure: a joint case–control study of melanoma in the UK and Australia. Br J Cancer 1998;77(3):505-10.

98. Schwartz JL, Wang TS, Hamlton TA, Lowe L, Sondak VK, Johnson TM. Thin primary cutaneous melanomas: associated detection patterns, lesion characteristics, and patient characteristics. Cancer 2002 Oct 1;95(7):1562-8.

99. Brady MS, Oliveria SA, Christos PJ, Berwick M, Coit DG, Katz J, et al. Patterns of detectionin patients with cutaneous melanoma. Cancer 2000 Jul 15;89(2):342-7.

100. McFarland SL, Schram SE. Physician skin examinations for melanoma screening. Cutis 2015 Sep;96(3):175-82.

101. Terushkin V, Halpern AC. Melanoma early detection. Hematol Oncol Clin North Am 2009 Jun;23(3):481-500.

102. Cherian P, Tait CP. Melanoma in private practice: Do dermatologists make a difference? Australas J Dermatol 2009 Nov;50(4):257-60.

103. Shellenberger R, Nabhan M, Kakaraparthi S. Melanoma screening: A plan for improving early detection. Ann Med 2016;48(3):142-8.

104. Federman DG, Shelling ML, Kirsner RS. Prevalence and correlates of skin cancer screening among middle-aged and older white adults in the United States. Am J Med 2010 Dec;123(12):e11.

105. Okhovat JP, Beaulieu D, Tsao H, Halpern AC, Michaud DS, Shaykevich S, et al. The first 30 years of the American Academy of Dermatology skin cancer screening program: 1985-2014. J Am Acad Dermatol 2018 Nov;79(5):884-891.e3.

106. Bandarchi B, Jabbari CA, Vedadi A, Navab R. Molecular biology of normal melanocytes and melanoma cells. J Clin Pathol 2013 Aug;66(8):644-8.

107. Gerstenblith MR, Shi J, Landi MT. Genome-wide association studies of pigmentation and skin cancer: a review and meta-analysis. Pigment Cell Melanoma Res 2010 Oct;23(5):587-606.

108. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970 Nov;172(5):902-8.

109. Landow SM, Gjelsvik A, Weinstock MA. Mortality burden and prognosis of thin melanomas overall and by subcategory of thickness, SEER registry data, 1992-2013. J Am Acad Dermatol 2017 Feb;76(2):258-63.

110. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005 Jan;41(1):45-60.

111. Fortes C, de Vries E. Non solar occupational risk factors for cutaneous melanoma. Int J Dermatol 2008 Apr;47(4):319-28.

112. Fitzpatrick TB. The validity and practicability of sun-reactive skin types I through VI. Arch Dermatol 1988 Jun;124(6):869-71.

113. Aalborg J, Morelli JG, Byers TE, Mokrohisky ST, Crane LA. Effect of hair color and sun sensitivity on nevus counts in white children in Colorado. J Am Acad Dermatol 2010 Sep;63(3):430-9.

114. Walter SD, Ashbolt R, Dwyer T, Marrett LD. Do larger people have more naevi? Naevus frequency versus naevus density. Int J Epidemiol 2000 Dec;29(6):1025-30.

115. Kontautiene S, Stang A, Gollnick H, Valiukeviciene S. The role of phenotype, body mass index, parental and sun exposure factors in the prevalence of melanocytic nevi among schoolchildren in Lithuania. J Eur Acad Dermatol Venereol 2015 Aug;29(8):1506-16.

116. Karimi K, Lindgren TH, Koch CA, Brodell RT. Obesity as a risk factor for malignant melanoma and non-melanoma skin cancer. Rev Endocr Metab Disord 2016 Sep;17(3):389-403.

117. Skowron F, Bérard F, Balme B, Maucort-Boulch D. Role of obesity on the thickness of primary cutaneous melanoma. J Eur Acad Dermatol Venereol 2015 Feb;29(2):262-9.

118. Avilés-Izquierdo JA, Molina-López I, Rodríguez-Lomba E, Marquez-Rodas I, Suarez-Fernandez R, Lazaro-Ochaita P. Who detects melanoma? Impact of detection patterns on characteristics and prognosis of patients with melanoma. J Am Acad Dermatol 2016 Nov;75(5):967-74.

119. Pennie ML, Soon SL, Risser JB, Veledar E, Culler SD, Chen SC. Melanoma outcomes for Medicare patients - Association of stage and survival with detection by a dermatologist vs a nondermatologist. Arch Dermatol 2007 Apr;143(4):488-94.

120. Geller AC, Koh HK, Miller DR, Clapp RW, Mercer MB, Lew RA (1992). Use of health services before the diagnosis of melanoma: implications for early detection and screening. J Gen Intern Med 1992 Mar-Apr;7(2):154-7.

121. Amrock SM, Meydani A. Trends and disparities in total-body skin examination: evaluating the national health interview survey, 2000-2010. JAMA Dermatol 2013 Mar;149(3):363-4.

122. Federman DG, Kravetz JD, Kirsner RS. Skin cancer screening by dermatologists: Prevalence and barriers. J Am Acad Dermatol 2002 May;46(5):710-4.

123. Kimball AB, Resneck JS. The US dermatology workforce: A specialty remains in shortage. J Am Acad Dermatol 2008 Nov;59(5):741-5.

124. Resneck JS, Kimball AB. Who else is providing care in dermatology practices? Trends in the use of nonphysician clinicians. J Am Acad Dermatol 2008 Feb;58(2):211-6.

125. Zalaudek I, Kittler H, Marghoob AA, Balato A, Blum A, Dalle S. Time required for a complete skin examination with and without dermoscopy. Arch Dermatol 2008 Apr;144(4):509-13.

126. Davis SA, Feldman SR, Fleischer AB Jr. Is the duration of skin disease visits decreasing in the United States? Dermatology Online Journal 2014 Dec 13;21(2).pii: 13030/qt449576gx.

127. Fischer M, Bergert H, Marsch WC. The dermatologic consultation. Hautarzt 2004 Jun;55(6):543-8.

128. Haenssle HA, Hoffmann S, Holzkamp R, Samhaber K, Lockmann A, Fliesser M. Melanoma thickness: the role of patients' characteristics, risk indicators and patterns of diagnosis. J Eur Acad Dermatol Venereol 2015 Jan;29(1):102-8.

129. Aldridge RB, Naysmith L, Ooi ET, Murray CS, Rees JL. The importance of a full clinical examination: assessment of index lesions referred to a skin cancer clinic without a total body skin examination would miss one in three melanomas. Acta Derm Venereol 2013 Nov;93(6):689-92.

130. Schneider JS, Moore DH, Mendelsohn ML. Screening program reduced melanoma mortality at the Lawrence Livermore National Laboratory, 1984 to 1996. J Am Acad Dermatol 2008 May;58(5):741-9.

131. Schwartz JL, Wang TS, Hamilton TA, Lowe L, Sondak VK, Johnson TM. Thin primary cutaneous melanomas - Associated detection patterns, lesion characteristics, and patient characteristics. Cancer 2002 Oct;95(7):1562-8.

132. Aneja S, Aneja S, Bordeaux JS. Association of increased dermatologist density with lower melanoma mortality. Arch Dermatol 2012 Feb;148(2):174-8.

133. Moran B, McDonald I, Wall D, O'Shea SJ, Ryan C, Ryan AJ. Complete skin examination is essential in the assessment of dermatology patients: findings from 483 patients. Br J Dermatol 2011 Nov;165(5):1124-6.

134. Chiaravalloti AJ, Laduca JR. Melanoma screening by means of complete skin exams for all patients in a dermatology practice reduces the thickness of primary melanomas at diagnosis. J Clin Aesthet Dermatol 2014 Aug;7(8):18-22.

135. Schmitt J, Seidler A, Heinisch G, Sebastian G. Effectiveness of skin cancer screening for individuals age 14 to 34 years. J Dtsch Dermatol Ges 2011 Aug;9(8):608-16.

136. Geller AC, Greinert R, Sinclair C, Weinstock MA, Aitken J, Boniol M. A nationwide population-based skin cancer screening in Germany: proceedings of the first meeting

of the International Task Force on Skin Cancer Screening and Prevention (September 24 and 25, 2009). Cancer Epidemiol 2010 Jun;34(3):355-8.

137. Hoorens I, Vossaert K, Pil L, Boone B, De Schepper S, Ongenae K. Total-Body Examination vs Lesion-Directed Skin Cancer Screening. JAMA Dermatol 2016 Jan;152(1):27-34.

138. Katalinic A, Waldmann A, Weinstock MA, Geller AC, Eisemann N, Greinert R. Does skin cancer screening save lives?: An observational study comparing trends in melanoma mortality in regions with and without screening. Cancer 2012 Nov;118(21):5395-402.

139. Stang A, Jöckel KH. Does skin cancer screening save lives? A detailed analysis of mortality time trends in Schleswig-Holstein and Germany. Cancer 2016 Feb;122(3):432-7.

140. US Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Ebell M, et al. Screening for Skin Cancer: US Preventive Services Task Force Recommendation Statement. JAMA 2016 Jul 26;316(4):429-35.

141. Gordon LG, Rowell D. Health system costs of skin cancer and cost-effectiveness of skin cancer prevention and screening: a systematic review. Eur J Cancer Prev 2015;24(2):141-9.

142. Schofield JK, Fleming D, Grindlay D, Williams H. Skin conditions are the commonest new reason people present to general practitioners in England and Wales. Br J Dermatol 2011 Nov;165(5):1044-50.

143. Toy EL, Vekeman F, Lewis MC, Oglesby AK, Duh MS. Costs, resource utilization, and treatment patterns for patients with metastatic melanoma in a commercially insured setting. Curr Med Res Opin 2015 Aug;31(8):1561-72.

144. Echeverría B, Bulliard JL, Guillén C, Nagore E. Indicators for the total number of melanocytic naevi: an adjunct for screening campaigns. Observational study on 292 patients. Br J Dermatol 2014 Jan;170(1):144-9.

145. Argenziano G, Zalaudek I, Hofmann-Wellenhof R, Bakos RM, Bergman W, Blum A. Total body skin examination for skin cancer screening in patients with focused symptoms. J Am Acad Dermatol 2012 Feb;66(2):212-9.

146. Argenziano G, Giacomel J, Zalaudek I, Apalla Z, Blum A, De Simone P. Twenty nevi on the arms: a simple rule to identify patients younger than 50 years of age at higher risk for melanoma. Eur J Cancer Prev 2014 Sep;23(5):458-463.

6. APPENDIX

Table 1. Number of nevi on the face

			95% Confidence Interval	
Number of nevi on the body	Mean number of nevi on the face	Std. Error	Lower Bound	Upper Bound
1.00 < 10	1.500	0.173	1.161	1.839
2.00 11 a 50	3.718	0.148	3.427	4.009
3.00 > 50	8.240	0.193	7.862	8.619

The predictive positive value was calculated with logistic regression.

Table 2. Sensitivity of the study of the 20 nevi on the arm

	≥50nevi on the body	<50nevi on the body
≥20 nevi on the arm	Sensitivity =59,8%	False Positive = 4,8%
< 20 nevi on the arm	False Negative =40,2%	Especificity=95,2%

Twenty nevi on the arms: a simple rule to identify patients younger than 50 years of age at higher risk for melanoma Giuseppe Argenzianoa , Jason Giacomeld , Iris Zalaudekb,e, Zoe Apallag , Andreas Blumh , Paola De Simonec , Aimilios Lallasa , Caterina Longoa , Elvira Moscarellaa , Danica Tiodorovic-Zivkovici , Jelica Tiodorovici , Dragan L. Jovanovici and Harald Kittlerf.

Table 3. Sensitivity nevi on the face

	≥50 nevi on the body	<50nevi on the body
≥8 nevi on the face	Sensitivity=59.1	False Positive=5.5
<8 nevi on the face	False Negative=40.9	Especificity=94.5

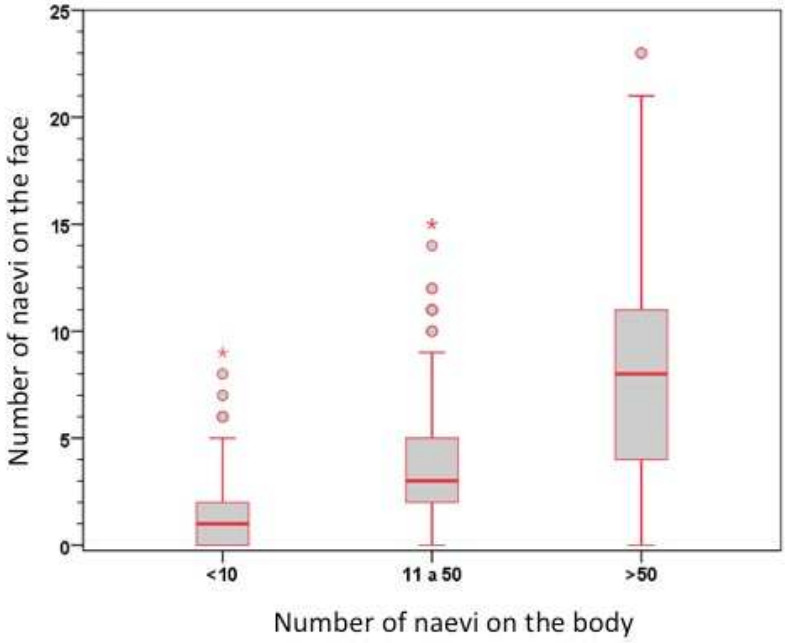

Figure 1. Boxplot correlation between nevi on the face and body

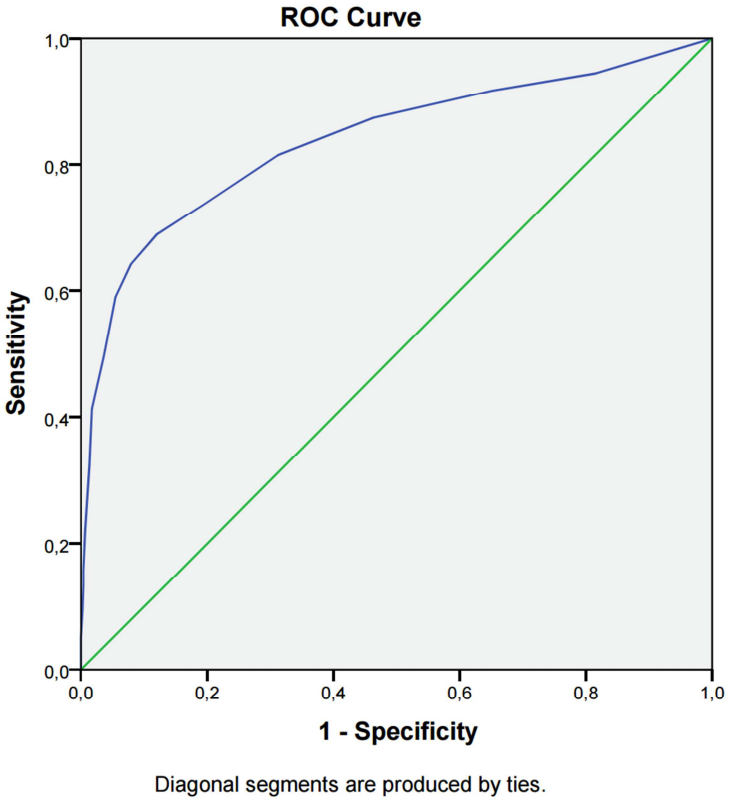

Figure 2. ROC curve

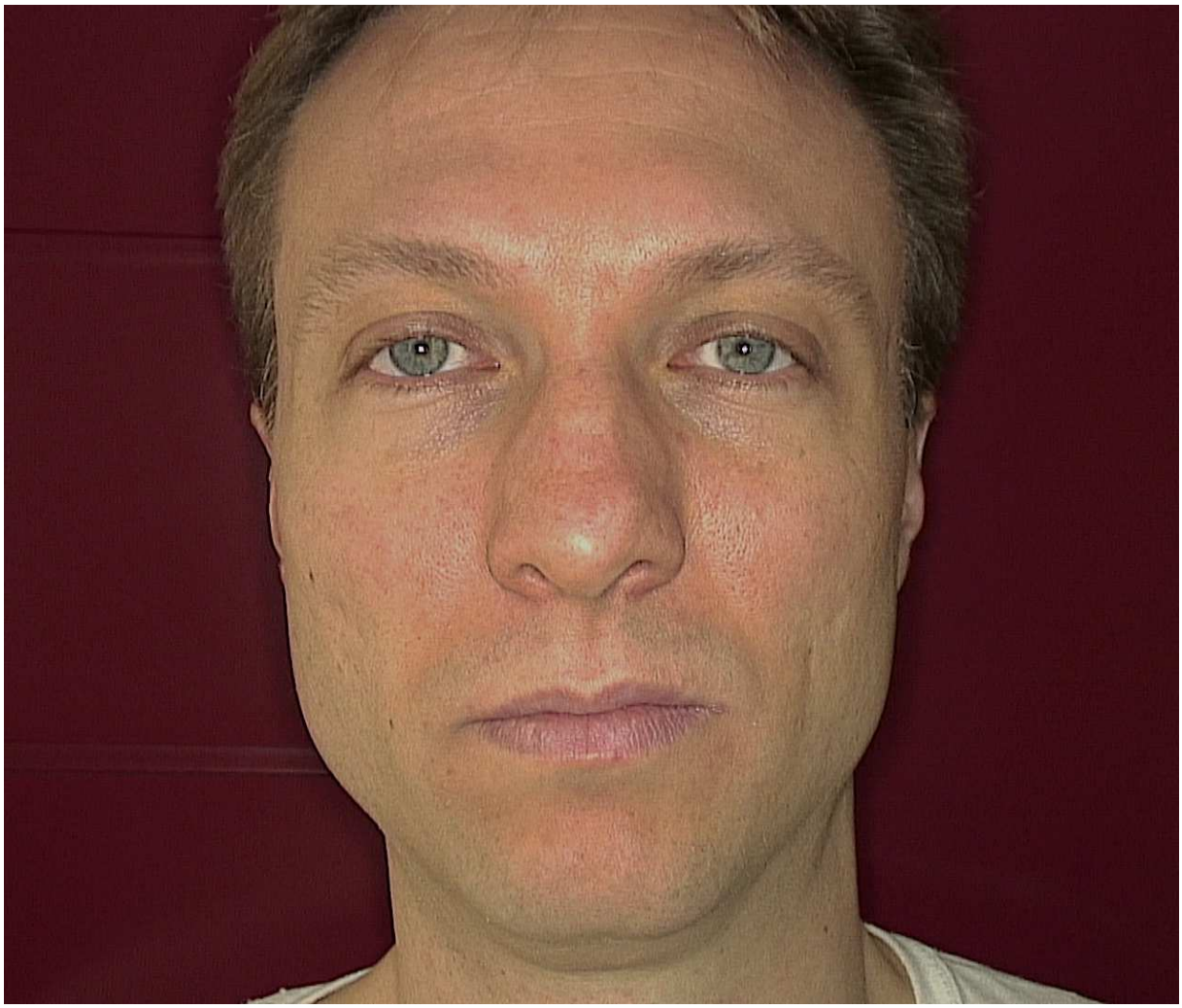

Figure 3. Individual with nevi on the face

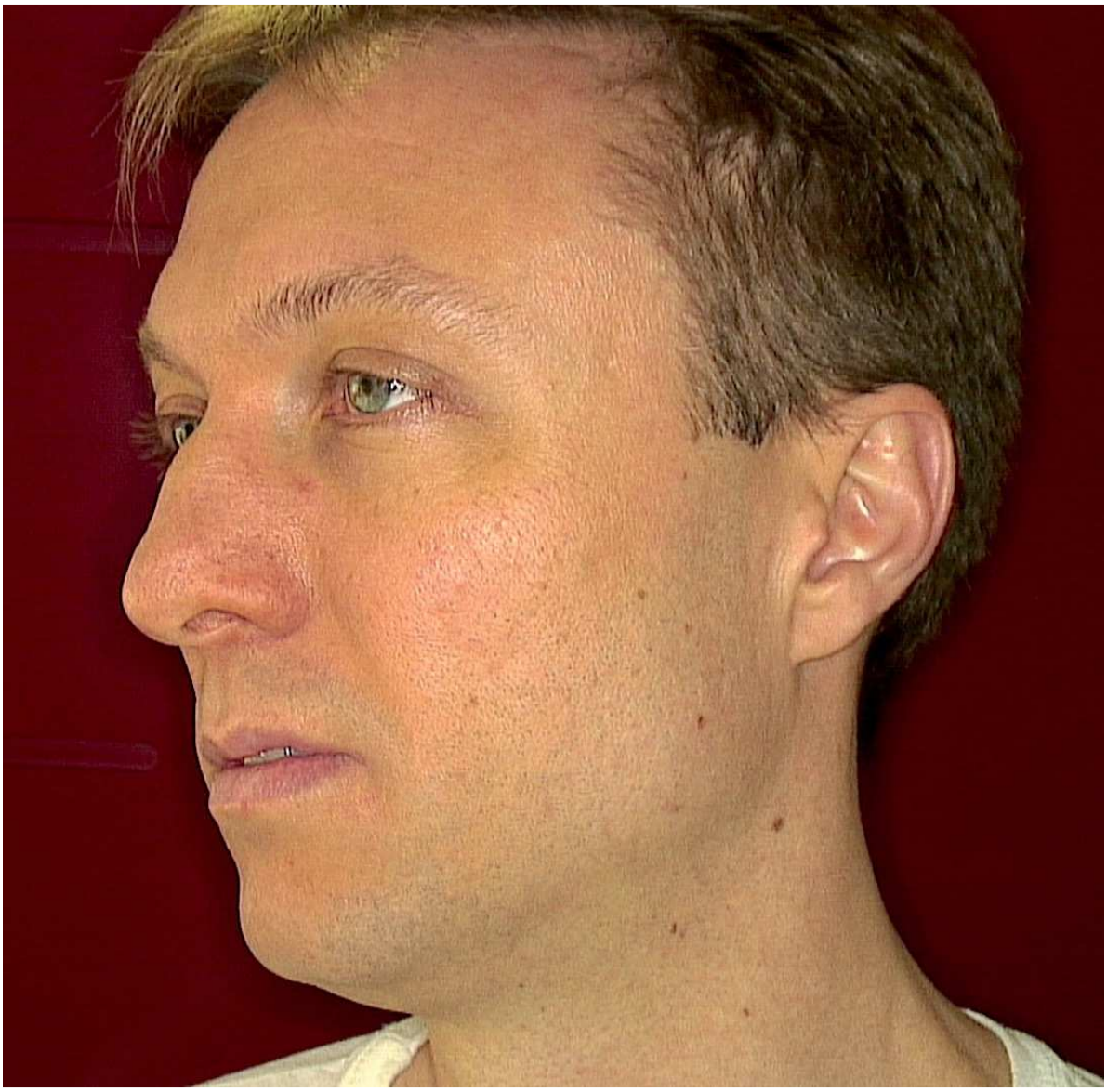

Figure 4. Face left

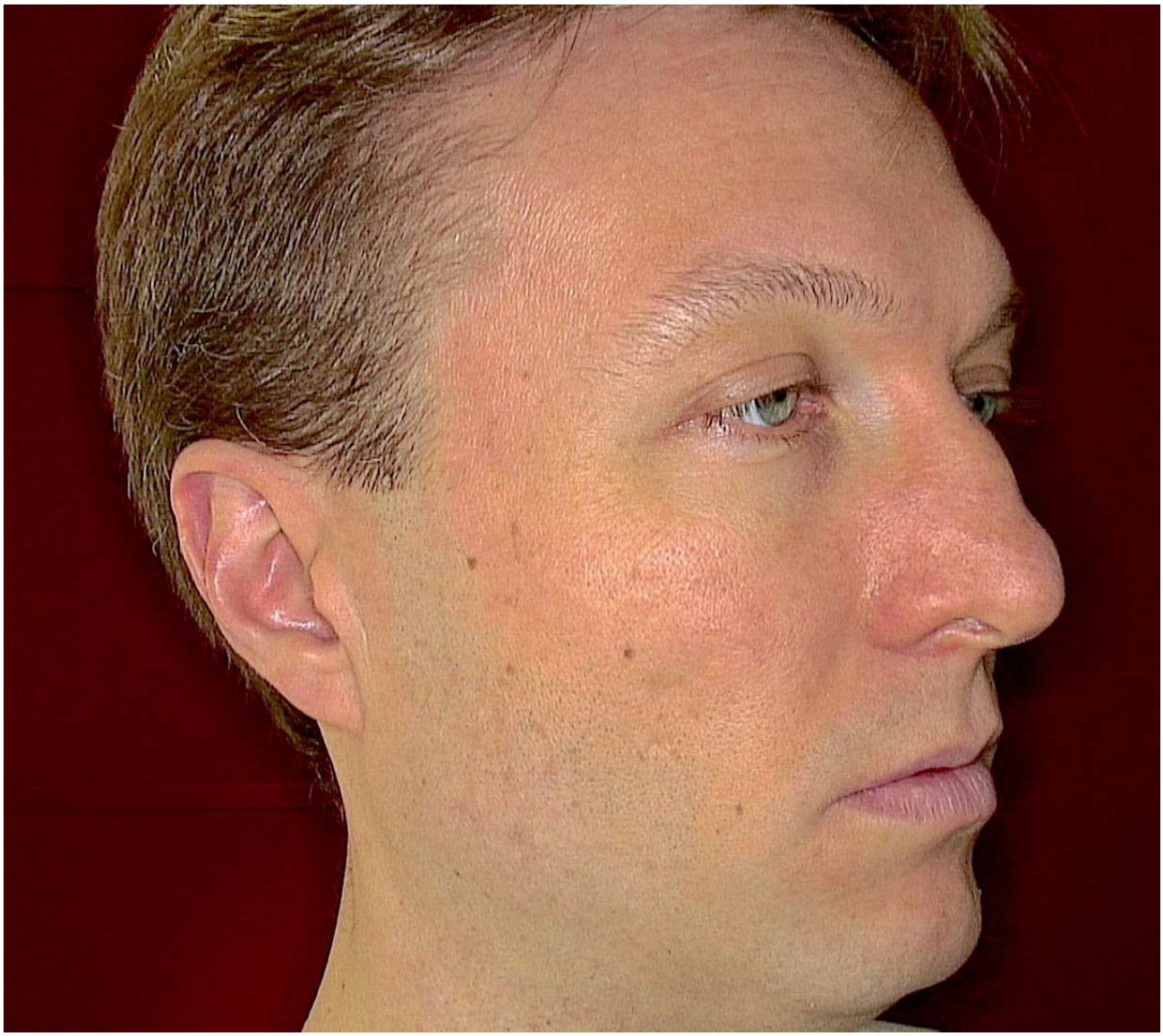

Figure 5. Face right

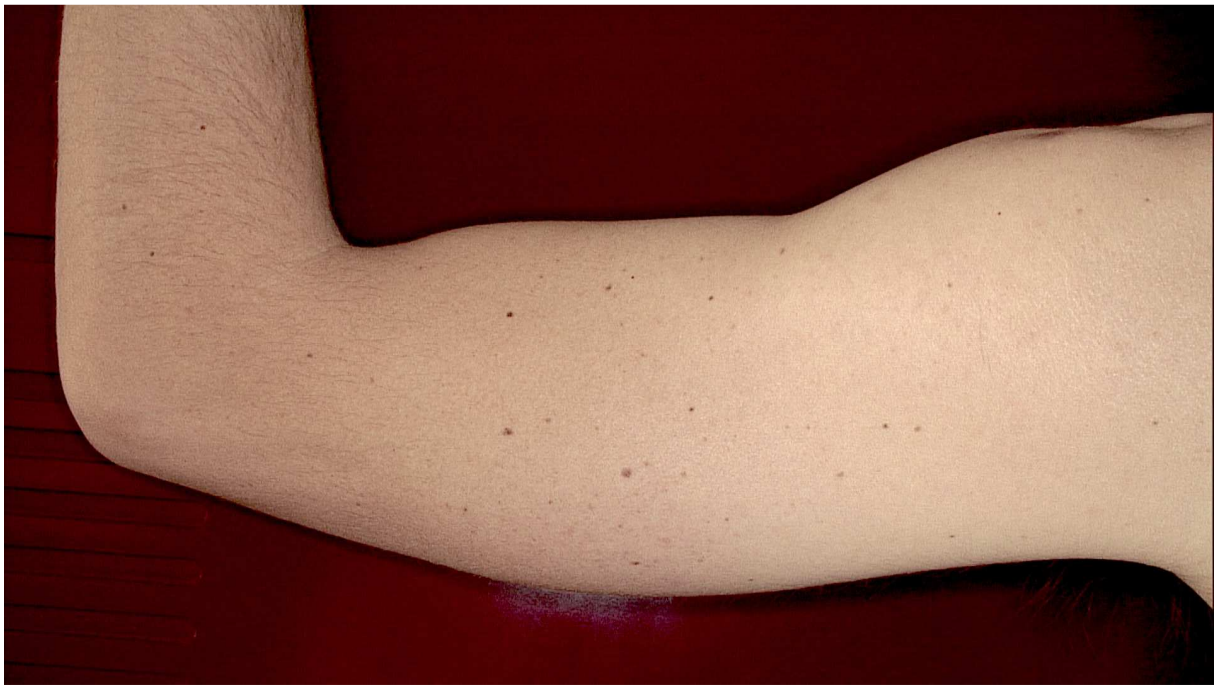

Figure 6. Right arm

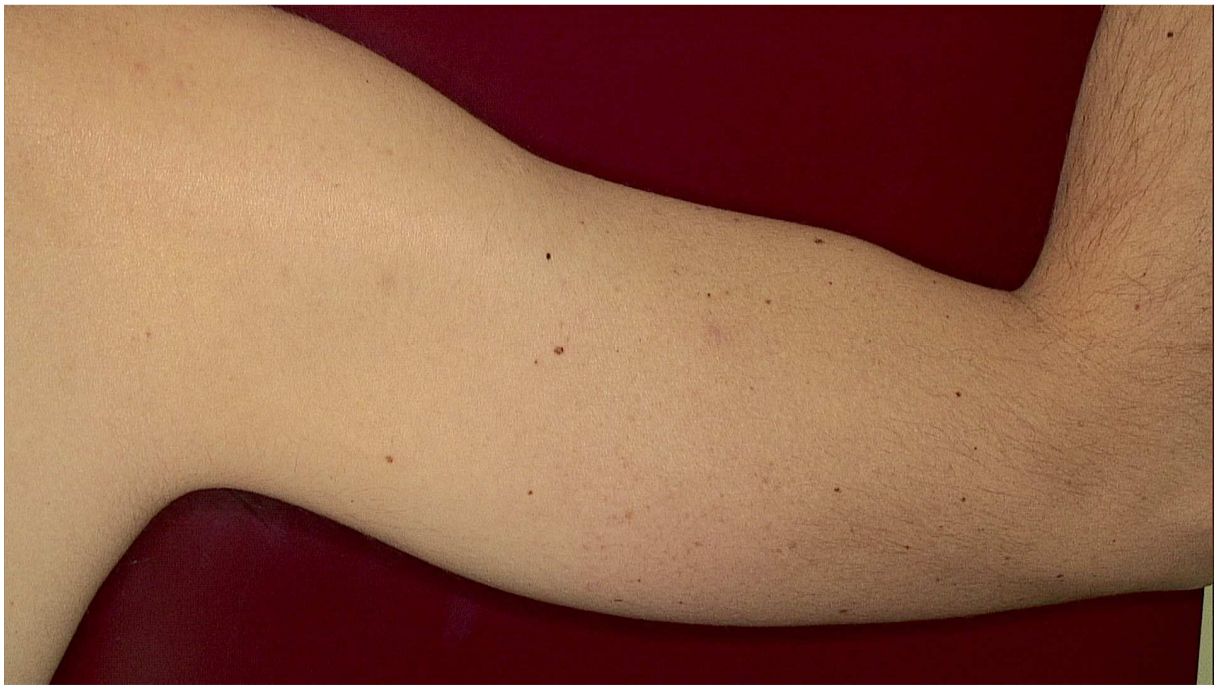

Figure 7. Left arm

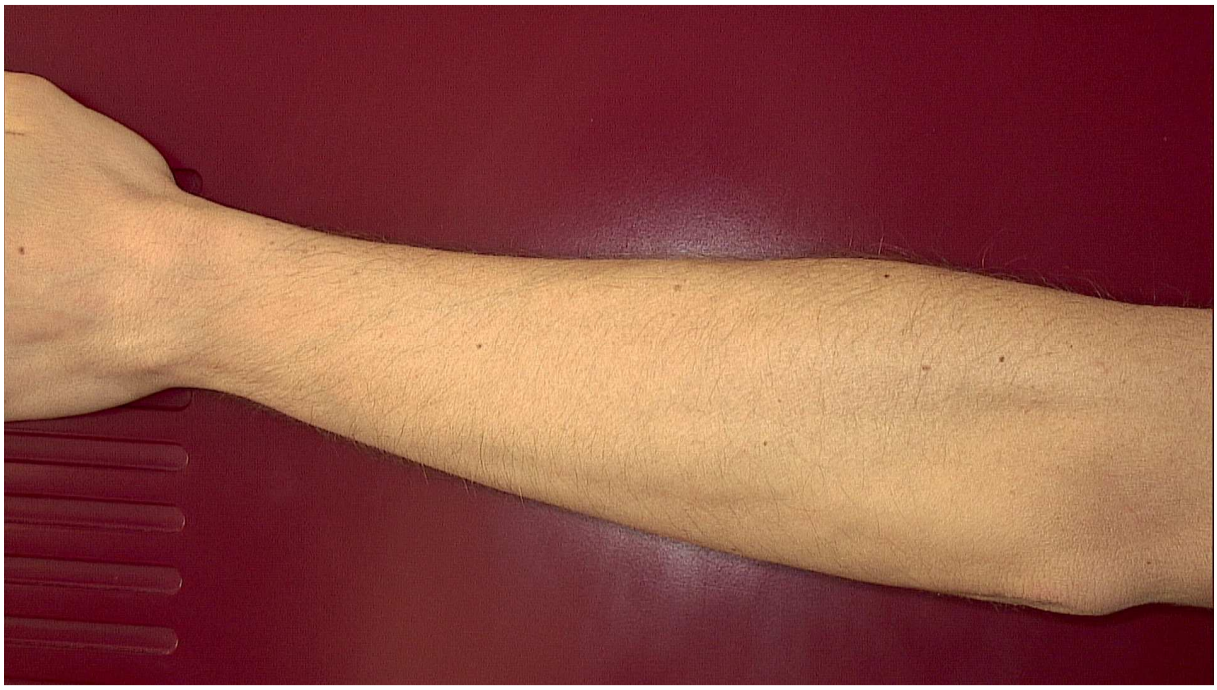

Figure 8. Right forearm

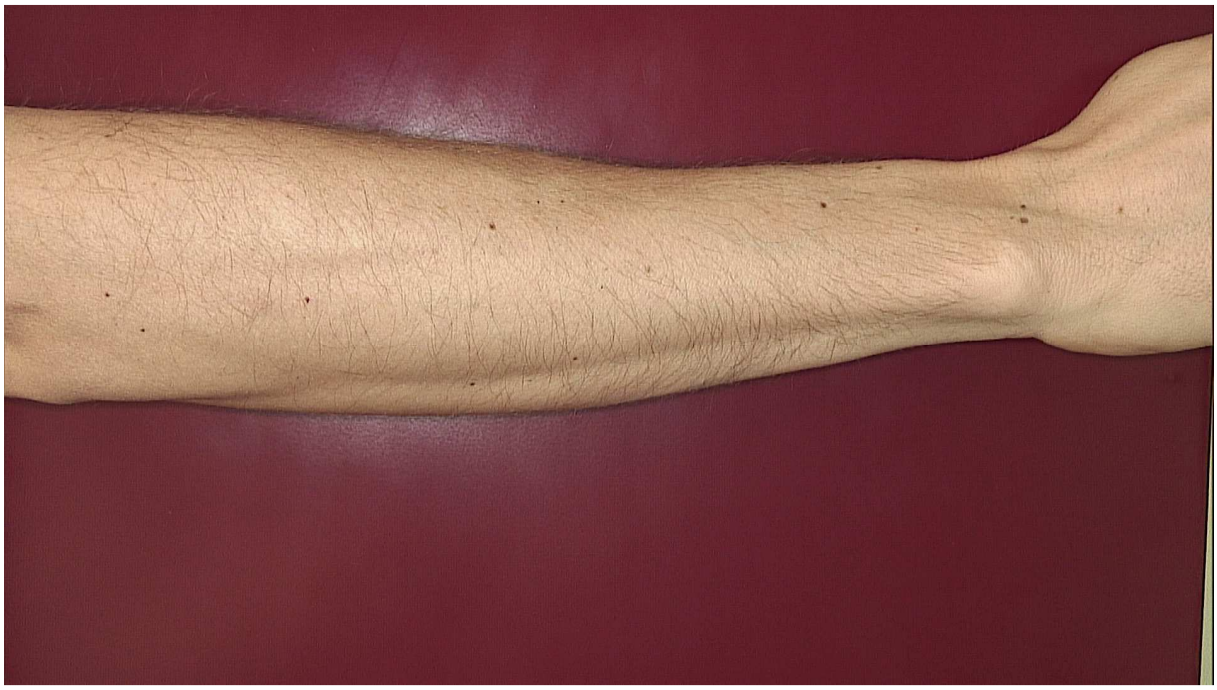

Figure 9. Left forearm

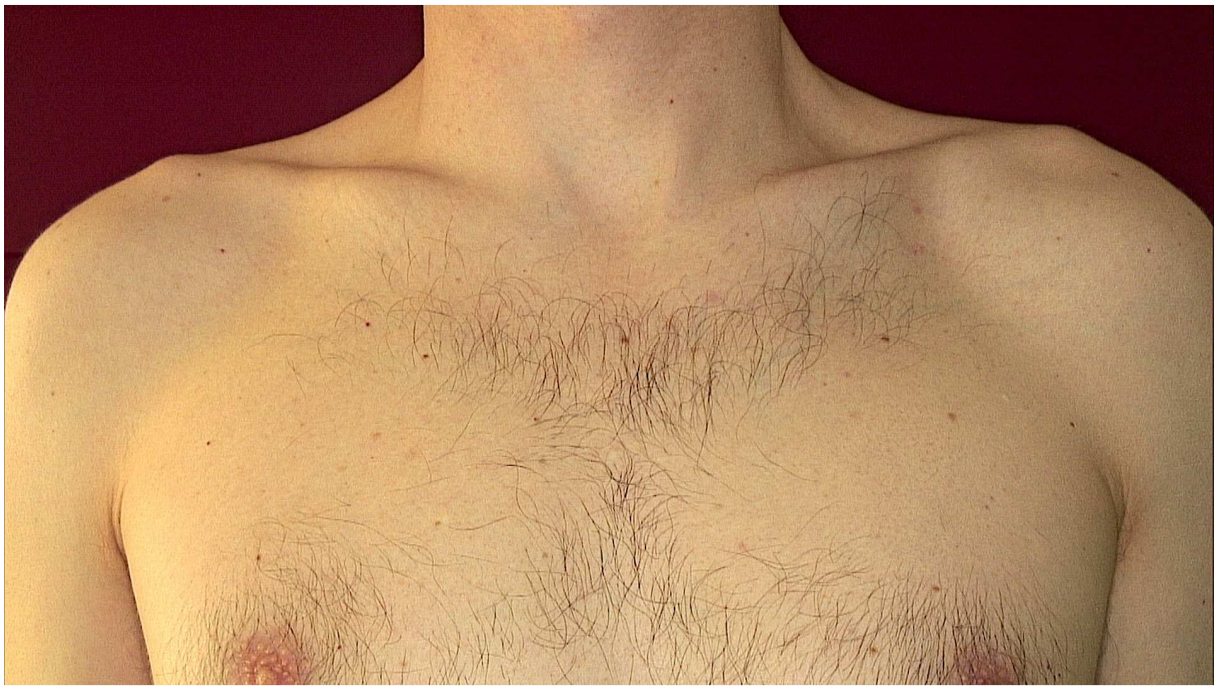

Figure 10. Chest

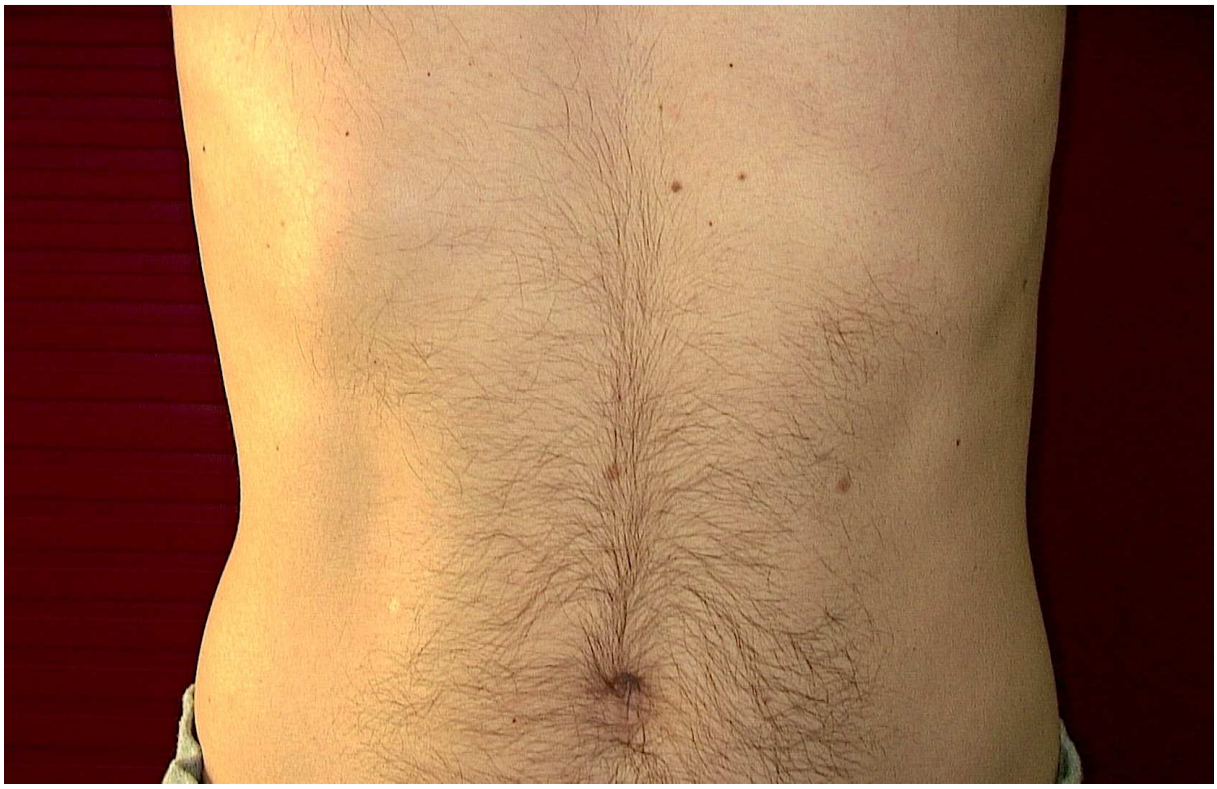

Figure 11. Abdomen

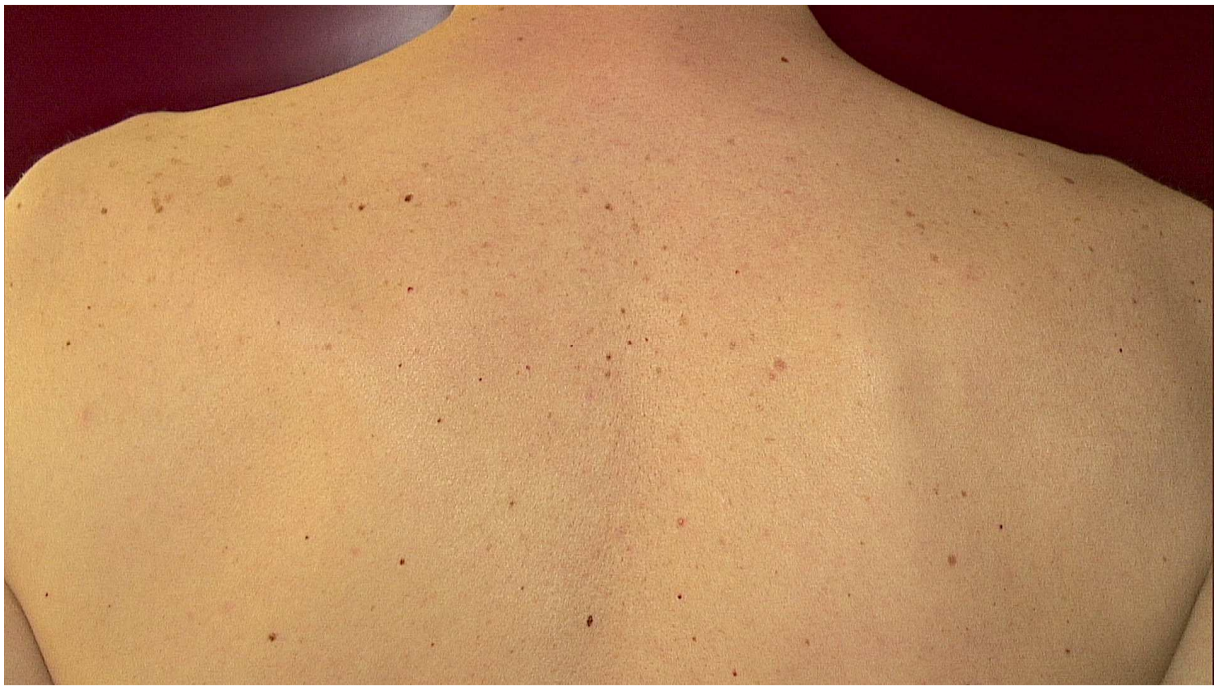

Figure 12. Upper dorsum

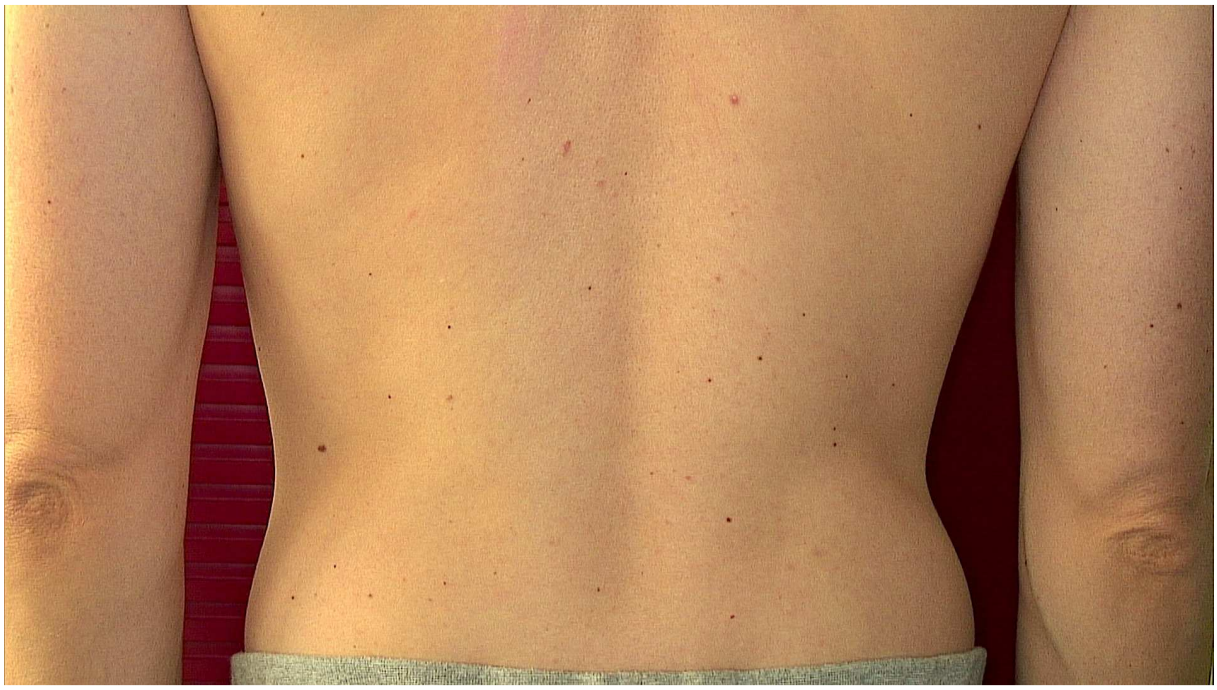

Figure 13. Lower dorsum