# Histological analysis of the dermal and hypodermal layers of the face and correlation with high-frequency 24 MHz ultrasonography and elastosonography

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ABSTRACT

Knowledge of the structure of the face is of fundamental importance. In fact, the face is treated in many areas of medicine, from dermatology, to maxillofacial, to ENT, to ophthalmology, *etc.* and anti-aging aesthetic treatments, and those for the resolution of blemishes are on the increase. For ethical reasons it is not possible to take biopsy samples for facial analysis in the aesthetic field. The main aim of this study was to demonstrate that a high-resolution bimodal ultrasound examination, combined with elastosonography, could be a valid tool for pre-treatment morphological evaluation. To achieve this goal, skin samples were taken from the forehead, zygomatic area, nasolabial fold, upper and lower lip from cadavers to histologically characterize their structure. Subsequently, these same areas were evaluated *in vivo* using conventional B-mode ultrasound with a 24 MHz high-frequency probe, and elastosonography. The data obtained with the different techniques were compared, in order to state that modern ultrasound techniques can provide similar histological information. The analysis showed that the superficial hypodermis presented a different shape and structure in the different areas, with the exception of the areas of the upper and lower lip, which appeared similar. With aging, the forehead and zygomatic area showed a volumetric increase in the superficial hypodermic layer, while the lip showed non-structural changes. The morphology of the nasolabial fold remained unchanged. When it is not possible to perform histological investigations on the face, to understand its characteristics and dynamics, ultrasound with a 24 MHz probe would seem to be the most suitable method, while elastosonography could be a valid method for evaluating the stiffness of the structural components.

**Key words:** face; aging; histology; high-resolution ultrasonography; elastosonography; dermis; hypodermis.

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## Introduction

The face is one of the most interesting objects in the field of anti-aging aesthetic medicine. It has a unique anatomical characterization compared to the rest of the body because, not only it is composed of different layers with complex continuity relationships, but it presents different conformations in different areas.[1] Its complexity is linked to its embryological development, given that the conformation of its superficial and deep adipose tissue compartments[2-4] is related to the embryological muscle development, and in particular to the development of the superficial musculoaponeurotic system (SMAS),[4] of the dense network of ligaments, of the extracellular matrix, of the blood and lymphatic vessels, and nerves[1,5] which, taken together, constitute the mosaic of the human face. The different structure of the various parts of the face is also linked to the multiple functions that these parts perform, functions ranging from the protection of the internal structures, to the auxiliary functions of the digestive, respiratory, visual and auditory systems, and, above all, the mimic function, which characterizes the human beings compared to other mammals. What differs in the different segments of the face is not only the structure, in terms of layers, but also the ultrastructure.[1,3,6] Considering the adipose compartments of the face, significant peculiarities were found compared to the subcutaneous adipose tissue present in other parts of the body, and described in detail by Sbarbati *et al*.[7] Applying the classification introduced in this study, most of the subcutaneous tissue of the face is fibrous white adipose tissue, that is, a tissue characterized by the presence of abundant collagen fibers, which form a basket-like structure surrounding clusters of adipocytes. Among the compartments with different structures, the malar and buccal (Bichat) fat pads should be mentioned, as they present scarce collagen fibers.[6] When choosing the most effective type of aesthetic treatment to solve the various problems of the face, it is not possible to ignore the structural varieties present in it. How useful can it be to make a highly concentrated collagen filler in a compartment that normally has a low collagen content? Schenck *et al*.[8] showed that injecting fillers into different compartments results in different behavior in those compartments, adding that a different structure reacts differently to the same treatment.

The choice of interventions to perform on a face cannot ignore the knowledge not only of the structure of the face, in terms of macroscopic anatomy, but also of the knowledge of the face in its structural characterization. Given the considerable inter- and intra-variability of the different subjects, the choice of each individual treatment must go through an anatomical pre-evaluation, performed directly on the person who will be treated. Considering the thicknesses of the anatomical structures under examination,[9] it is important to detail as accurately as possible, in particular the first millimetres of each area. Since for ethical reasons, it is not possible to take biopsy samples for facial analysis in the aesthetic field, the main aim of this study was to demonstrate that a high-resolution bimodal ultrasound examination with a 24 MHz probe combined with elastosonography could be a valid tool for pre-treatment anatomical evaluation.

## Materials and Methods

This study was conducted in full compliance with the ethical norms and standards in the Declaration of Helsinki.

Five facial points were chosen for anatomical and morphological evaluation:

- the frontal region, above the eyebrow arch, 2 cm medial to the temporal line;
- the zygomatic region, in front of the zygomaticotemporal joint, 1 cm above the zygomatic arch;
- the nasolabial fold;
- the region of upper lip, between the nasolabial fold and the philtral column;
- the lateral region of the lower lip.

Histological images of these 5 areas were obtained from cadaveric specimens. Cadaver samples were left for 12 h at room temperature to allow complete thawing prior to collection of skin samples. The 5 areas were dissected. Samples were fixed in 10% formalin for 24 h. For subsequent evaluations, samples were washed 3 times for 10 min with phosphate buffered solution 1x (PBS, pH 7.4), post-fixed with 3% paraformaldehyde, and washed again 3 times for 10 minutes with PBS. Then the samples were dried manually with paper, embedded in optimal temperature (OCT; -24°C), and cryosectioned with cryostat to obtain 14 µm sections. After 24 h, half of the slices were washed with PBS 1x, stained with Meyer's hematoxylin (H&E, Bio-Optica, Milan, Italy) for 30 s, washed in running water, stained with 1/10 eosin (H&E, Bio-Optica) for 10 s, and washed with distilled water. The remaining slices were washed with PBS 1x, stained with Alcian Blue (Merck KGaA, Darmstadt, Germany) for 30 min, washed with distilled water, stained with Fast Red solution (H&E, Bio-Optica) for 20 min, and washed with distilled water. Slides were dehydrated with increasing concentration of alcohol (60, 80, 95, 100%, each for 10 min), ending with a double wash in xylene for 10 minutes. Finally, slides were mounted whit Entellan (Merck KGaA), dried, and gently cleaned with ethanol. Slides were examined by light microscopy using an Olympus BX-51 microscope (Olympus, Tokyo, Japan), equipped with a JVC DKY-F58 CCD digital camera (Yokohama, Japan).

Subsequently, we performed a retrospective chart review of 24 subjects scheduled for soft tissue filler injections. The subjects were 23 females and 1 male, aged between 31 and 66 years. Demographics are detailed in Table 1. The 5 areas were investigated in all 24 subjects. All subjects had no undergone soft tissue filling treatments within the past 5 years, nor any facial surgical procedures that could have compromised the integrity of their facial anatomy. The images considered in this study were obtained for pre-treatment evaluation. An informed consent statement for data use was obtained from all 24 patients.

A conventional B-mode ultrasound was performed to evaluate the morphology of the different soft tissues present in these five points, and the anatomical correlations between them. A LOGIQ E10 (General Electric Co., Italy) with a high-frequency 24MHz probe was used. Ultrasound gel was applied to the skin during acquisition to achieve an acoustic interface, and prevent local artefacts related to the interposition of the air. Immediately after recording the B-mode ultrasound, elastosonography with a 15Mhz probe was performed to evaluate the structural mechanical properties of the soft tissues.

The subjects were positioned seated on an outpatient couch with the backrest inclined at 45 degrees to promote a comfortable and relaxed position during all measurements. Relaxation was essential to record the elasticity of the tissues without alterations

**Table 1.** Demographics of the patients.

| Age (years) | Subjects (number) | Previous treatments (>5 y) |
|---|---|---|
| ≤40 | 2 | 1 |
| 41-50 | 4 | 2 |
| 51-60 | 13 | 9 |
| ≥61 | 5 | 4 |

related to possible contractures of the facial muscles. Since it is known that elastosonography is operator-dependent, particular attention was paid to the pressure exerted with the probe on the underlying anatomical structures, to avoid image artefacts linked to excessive local pressure. Moreover, the probe was held perpendicular to the skin surface and light sequential pressures were performed with slow movements (<1 action/s) of approximately 1-2 mm amplitude to ensure optimal acquisition, avoiding stiffening induced by tapping the probe on the skin.

The images were re-processed to highlight the different structural components using the ImageJ.JS software (National Institute of Mental Health, Bethesda, MD, USA). In particular, the "Image - Color - Divide Channels" function was used for the elastosonographic images. This function divides the original image into three images, which represent the blue, green, and red components of the original image. The red components are the rigid components, such as the dermis, and the thicker connective fibers. The green components are the semi-rigid components, such as connective fibrils. The blue components are the soft components, i.e. those with greater elasticity, such as adipose tissue and elastic fibers.

All the acquired and reprocessed images were subjected to evaluation, as well as comparison with the histological images of the 5 areas. Cadaveric specimens were available only from subjects aged ≥50 years. Thus, in the evaluations, we considered the effect of aging on the skin structure of cadavers and compared and correlated histological images with ultrasound and elastographic data from 18 of the 24 subjects of the study, aged ≥50 years.

For the evaluations of the thicknesses of the dermis and hypodermis of the different areas, the subjects were divided into 3 groups: <50 y (n1=6), 51-60 y (n2=13), >60 y (n3=5). Data were expressed as mean±SD. One-way analysis of variance (ANOVA) was used to compare the mean thickness values between the studied groups. To evaluate the differences in thickness of the different areas of the face in the individual groups, the Student's *t*-test with Bonferroni's correction was used. All *p*-values were two-tailed, and considered significant at $p \leq 0.05$.

## Results

In general terms, for all 5 areas, in the ultrasound image, the skin appeared as a series of lines and bands in various shades of grey, black and white, which corresponded to the different layers of the skin. The epidermis looked as a hyperechoic line 0.12-0.13 mm thick. The dermis corresponded to a less bright homogeneous hyperechoic band of variable thickness depending on the anatomical location and age (Table 2), while the subcutaneous layer appeared as a hypoechoic layer representative of adipose lobules, with the presence of hyperechoic fibrous septae (Figure 1). Three different structural patterns were identified:

1. area with a very thin subcutaneous layer - frontal region;
2. area with abundant subcutaneous layer - zygomatic region and lateral portion of the nasolabial fold;
3. area with practically, but not completely absent subcutaneous layer - regions of the upper and lower lip.

While maintaining these structural schemes, the dimensional relationships and, consequently, the morpho-functional characteristics, were different in relation to the age of the subjects considered. In subjects of advanced age or with intense actinic damage, a hypoechoic layer called low echogenicity subepidermal band was observed between the dermis and epidermis. This band was a probable ultrasound manifestation of elastosis and edema in the papillary dermis.

**Table 2.** Dermis thickness of cheek and upper lip.

| Age (years) | Dermis thickness (mm) Cheek | Dermis thickness (mm) Upper lip | Mean difference (mm) | *p** |
|---|---|---|---|---|
| ≤50 | 2.0±0.3 | 3.4±0.5 | 1.4 | 0.012 |
| 51-60 | 1.9±0.3 | 3.5±0.4 | 1.6 | <0.0001 |
| ≥61 | 2.1±0.3 | 3.7±0.7 | 1.6 | 0.014 |

*2-tailed t-test with paired samples.

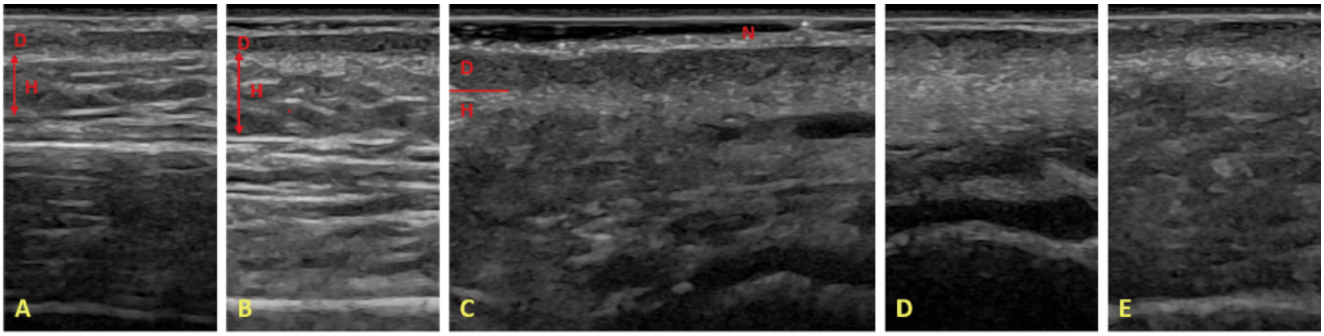


**Figure 1**. High-frequency 24 MHz ultrasonography of the first centimetre of the forehead (**A**), zygomatic area (**B**), nasolabial fold (**C**), upper lip (**D**), and lower lip (**E**) of a 59-year-old subject. The anatomical structure of all 5 areas is the same, as is their response to ultrasound. The thicknesses appear different, especially in the dermis and hypodermis. The structure of the hypodermis differs substantially between different areas. In **A**, **B** and **C** the demarcation between dermis and hypodermis is clear, while it is not in **D** and **E**. The superficial hypodermis is clearly distinguished from the underlying structures only in **A** and **B**. It is interesting to note how the nasolabial fold (C) separates two areas: the cheek and the upper lip, which present a completely different morphology. D, dermis; H, hypodermis; N, nasolabial fold.

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## Frontal region

This region was characterized by the presence of a scant subcutaneous layer (1,6 mm), which seemed to consist of small adipose lobules well encapsulated in robust connective fragments. The latter ran parallel to the skin surface. Under the adipose tissue was the occipitofrontalis muscle, directly connected to the periosteum (Figure 2). Forehead wrinkles were not evident in the subjects considered. With age, a thickening of the superficial hypodermis, and an alteration in the organization of the collagen fibers were noted (Figure 3) (thickness of the superficial hypodermis: subjects <50 y = 1.3±0.5 mm; subjects 51-60 y = 1.3±0.5 mm; subjects >60 y = 1.5±0.4 mm; ANOVA analysis revealed no significant differences among groups, *p*=0.63). Structurally, there was an increase in the soft components and a reduction in semi-rigid components (Figure 4).

## Zygomatic region

In this region the superficial hypodermis was well represented and consisted of small adipose lobules inserted in a network of thin but dense collagen fibers, arranged in several layers to form a true stratified matrix. Below the hypodermic layer, the structure of the SMAS was evident. It was characterized by very dense, coiled muscles and collagen fibers. Below the SMAS there was an additional layer of adipose tissue completely different from the superficial hypodermis, with thin collagen fibers distributed throughout the tissue. Thick collagen fibers enveloped the entire structure and separated this layer from both the upper SMAS and the underlying bone structures (Figure 5). As regards aging, the same trend observed for the forehead was highlighted (Figure 6) with variations at the level of superficial hypodermis, in particular, and of the entire connective structure (superficial hypodermis thickness: subjects <50 y = 2.3±0.7 mm; subjects 51-60 y = 2.3±0.7 mm; sub-

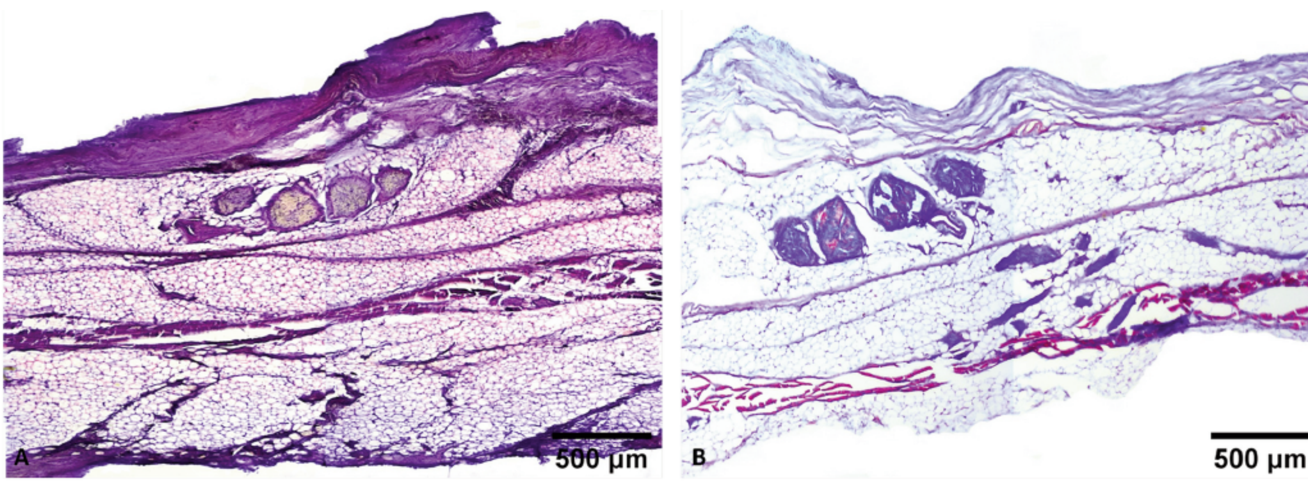


**Figure 2**. Serial light microscopy images of hematoxylin and eosin stained sections (**A**) and Alcian blue and fast red stained (**B**) sections of the forehead. The stretches of connective tissue parallel to the skin and surrounding the lobules of adipose tissue are evident.

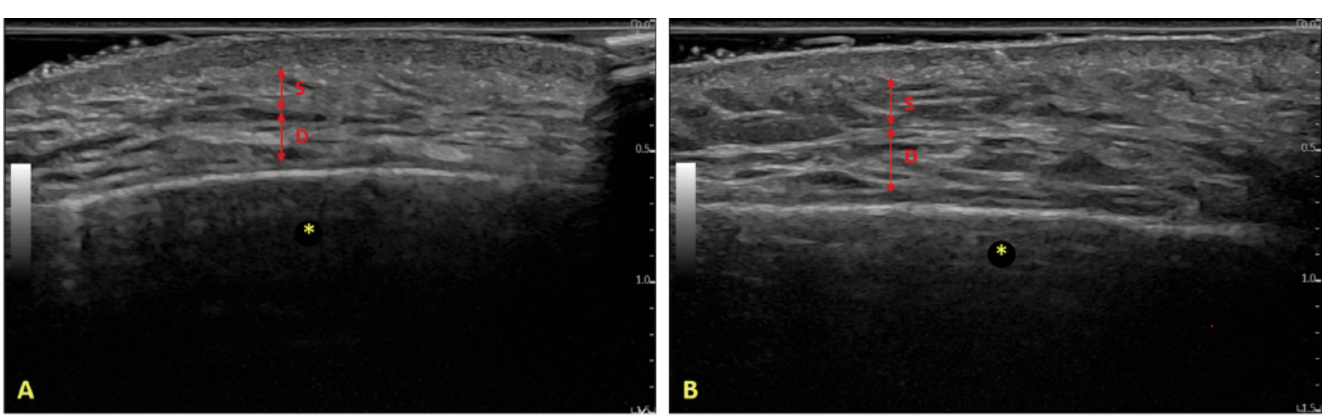


**Figure 3**. Ultrasound analysis makes it possible to highlight that the structural model of the forehead is similar between individuals of different ages. The peculiar differences concern the superficial and deep adipose compartments which seem to thicken when comparing young and elderly subjects. Here, the images obtained in a 31-year-old subject (**A**), and in a 65-year-old subject (**B**) are presented. D, deep hypodermis; S, superficial hypodermis; *bone.

jects >60 y = 2.6±0.7 mm; ANOVA analysis revealed no significant differences among groups, $p$=0.911); however, the structure with a prevalence of semi-rigid fibers was the same for all the subjects considered.

## Upper and lower lip regions

The structure of these two areas appeared similar (Figures 7 and 8). The entire hypodermic area appeared as a single, very dense band of collagen fibers with very thin fibrous tie rods that intertwined with the dermal fibers. Below the hypodermis there was a very thin cleavage plane (<1 mm thick) consisting of adipose tissue, placed particularly deep and adhering to an underlying muscular layer. The type of mesh of the hypodermic band appeared to be almost non-deformable and seemed to constitute a single functional unit. With age, the non-deformable structure changed and non-homogeneous areas were highlighted. However, the structural pattern was not altered and for all the subjects considered, a prevalence of the semi-rigid component of collagen was detected, compared to both the soft and the rigid components (Figure 9).

## Nasolabial fold

This line delimits two areas of the face that are structurally extremely different from each other (Figure 10). The labial region has already been described. Below the line, the compact subcutaneous layer of the upper lip region continued to present thicker and stiffer connective tissue components. Underneath this connective

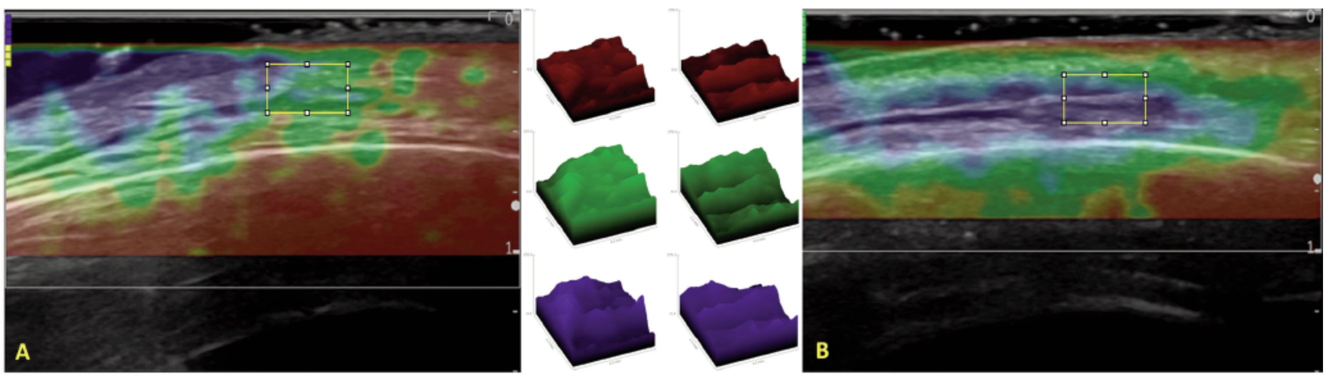


**Figure 4**. The elastosonographic analysis of the forehead shows that, although the skin structure is the same, individuals of different ages have different rigid (red), semi-rigid (green) and soft (blue) components. The distribution of these components is related to the degree of aging. On the front, the variation in the average percentage level of the semi-rigid components is dominant. Here is the elastography data of the two subjects in Figure 3. **A**) For the 31-year-old subject, the percentage of pixels in the selection box (out of a total of 255 pixels) corresponding to red (soft) components was 32.3%, that corresponding to the green (semi-rigid) components was 44.6%, and that corresponding to the blue (hard) components was 23.4%. **B**) For the 65-year-old subject, the percentage of pixels in the selection box (out of a total of 255 pixels) corresponding to the red (soft) components was 37.2%, that corresponding to the green (semi-rigid) components was 29.5%, and that corresponding to the blue (hard) components was 21.2%.

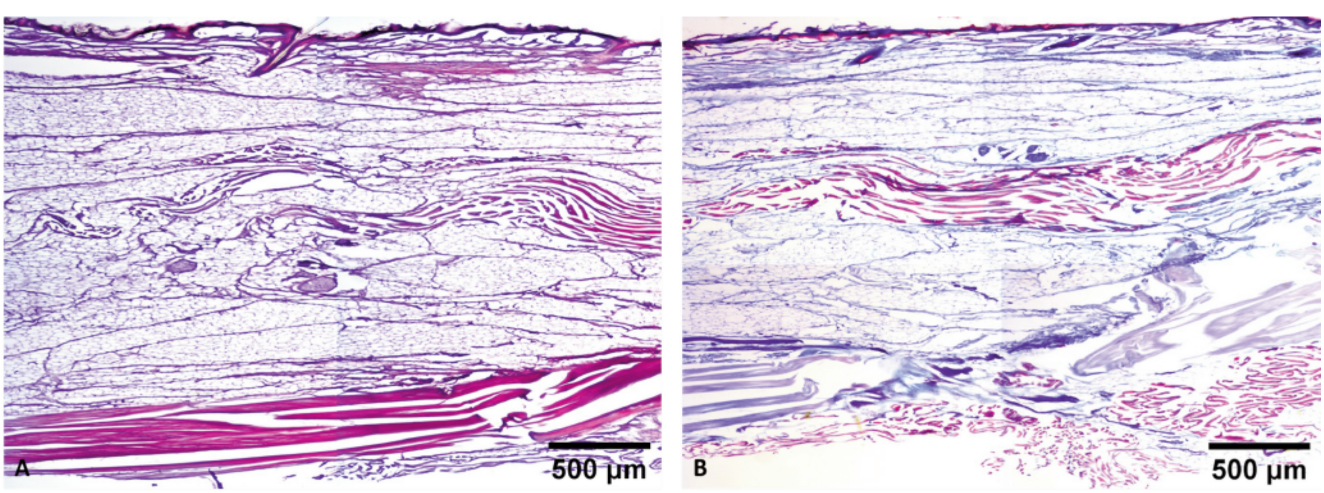


**Figure 5**. Serial light microscopy images of hematoxylin and eosin stained sections (**A**) and Alcian blue and fast red stained sections (**B**) of the zygomatic area. Note the stratified structure of this area and the important structural differences between the superficial hypodermal tissue and the deep hypodermal tissue beneath the SMAS. In the deep hypodermic tissue, the adipocytes appear larger in size. Thick fibers of connective tissue encapsulate this layer between the SMAS and the underlying muscle.

tissue was the superior labial artery. Moving laterally, the subcutaneous compact layer bifurcated, with the main component moving into the deep layers of the cheek, and into which the SMAS structure was embedded. Only a small component of the compact subcutaneous layer remained on the superficial layers of the cheek dermis (Table 2; significant difference in thickness for all ages). A superficial adipose lobule poor in connective structures, with oval-shaped lobules, was inserted between the two arms. Below the SMAS and the connective tissue in which it was inserted, a deep fat compartment was noted, with spherical lobules larger than those observed in the superficial compartment. At the superficial level, the presence of dense connective excerpts arranged parallel to the skin surface was noted. These structures appeared absent at a deep level, where, instead, there were thin fibers gathered to form support structures orthogonal to the skin surface (Figure 11). As regards the nasolabial fold, although it seems to accentuate with age, no particular variations in the structural pattern were highlighted in the subjects considered.

## Discussion

The study conducted with high resolution ultrasound equipment proved to be extremely useful for the analysis of facial structures, with particular reference to the superficial layers. Our analysis focused on the dermal and subcutaneous layers that were examined in detail in the five anatomical areas studied, and the ultrasound and elastosonographic images were compared with the histological images. Ultrasonography provides similar histological information, while elastosonography provides similar histochemical information.

The different organization of the subcutaneous tissue in the facial compartments described by Bertossi *et al.*,[6] Wan *et al.*,[3] and Kruglikov *et al.*[1] was confirmed. Different compartments had their own microstructure, particularly evident in the composition of the extracellular matrix.[1,6]

In this study it was possible to recognize some specific patterns for the various compartments and, thanks to the ultrasound analysis, to detail the distribution of both the connective septa that organize and orient the subcutaneous tissue, and the subsequent modifications, due to aging, of these septa, and fat layers. Finally, more in-depth knowledge of these structures allowed us to hypothesize their functionality, correlated to both wear and aging, phenomena linked to a relationship of dependence between them.

### Frontal region

The first pattern, identified at the level of the forehead, consisted in younger subjects in a thin subcutaneous layer with thick par-

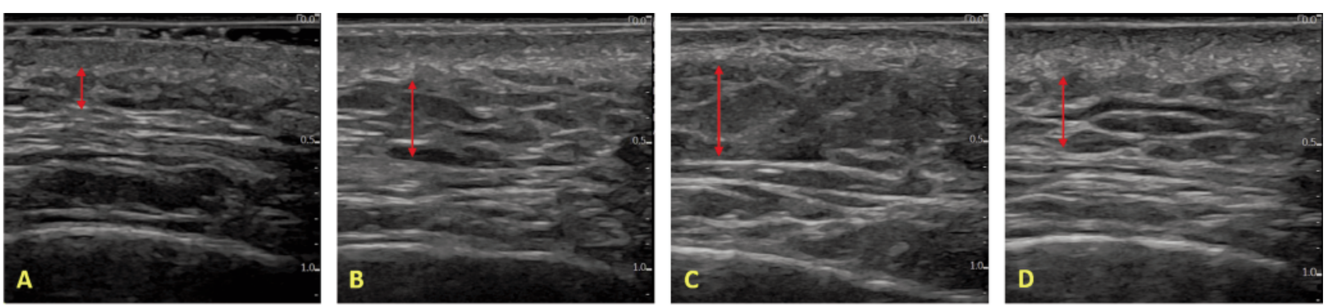


**Figure 6**. The comparison of the ultrasound image of the zygomatic area among a 31-year-old subject (**A**), a 40-year-old subject (**B**), a 53-year-old subject (**C**), and a 63-year-old subject (**D**) highlights the structural variation in particular of the superficial hypodermis that thickened (red arrows). An alteration of the connective structure is particularly evident under the superficial hypodermis, in the deeper fat compartments.

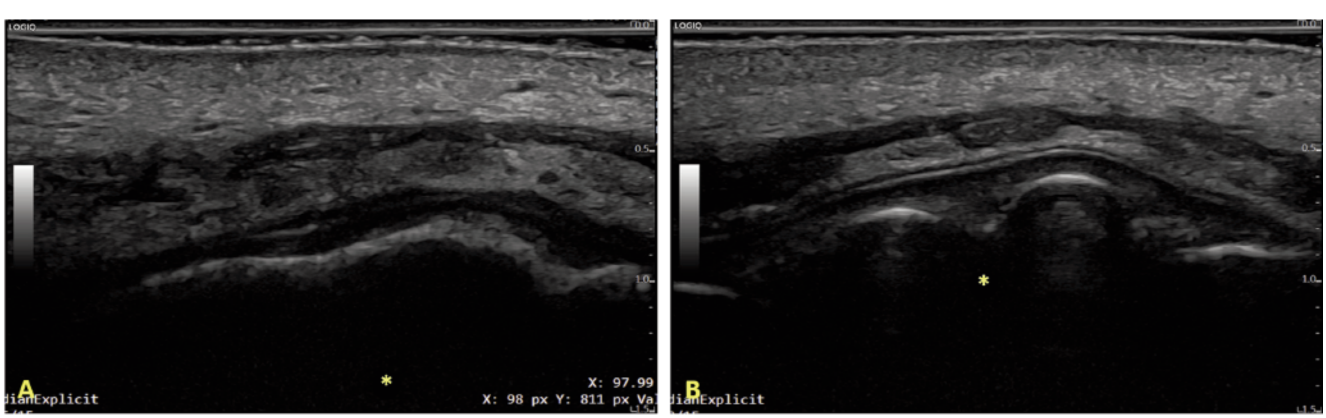


**Figure 7**. Ultrasound analysis of a portion of the upper (**A**) and lower (**B**) lip in a 46-year-old subject. The structural model of the two areas is similar. The thick hyperechoic band beneath the epidermis is a complex structure with extracellular matrix composed of dense connective tissue. As highlighted in Figure 1, there is not clear demarcation between the dermis and hypodermis. *Buccal cavity.

allel septa, arranged to create a "rosary organization" of the layer (Figure 3). This organization is linked to the type of motility of the forehead, which manifests itself on the single vertical plane. This movement is also compatible with the direction of wrinkle formation (which is opposed by the effect of the pneumatic action of the adipose tissue present), which occurs orthogonally to the movement, i.e. horizontally along the forehead.[10,11] The difference between the structure of the young and that of the elderly may be linked to the presence of the horizontal wrinkles. Morphologically, the wrinkles are characterized by a fibrous thickening at the dermo-hypodermic junction, and by a consequent increase in overall rigidity, correlated to an increase in the rigid components pre-

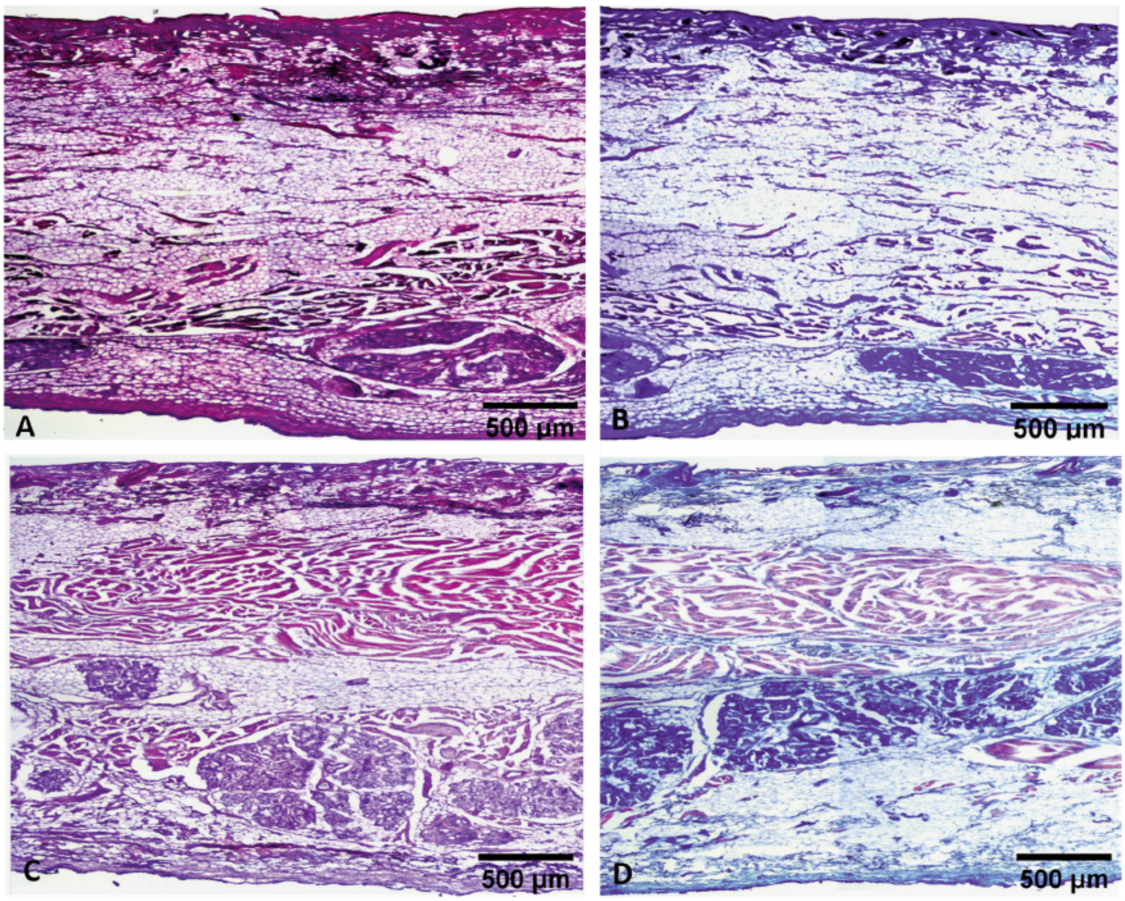


**Figure 8**. Serial light microscopy images of hematoxylin and eosin stained sections (**A**), Alcian blue and fast red stained sections of the upper lip (**B**), hematoxylin and eosin stained sections (**C**), and Alcian blue and fast red stained sections of the lower lip (**D**). The dermis and superficial hypodermis have a similar structure, extremely fibrous, due to the massive presence of connective tissue.

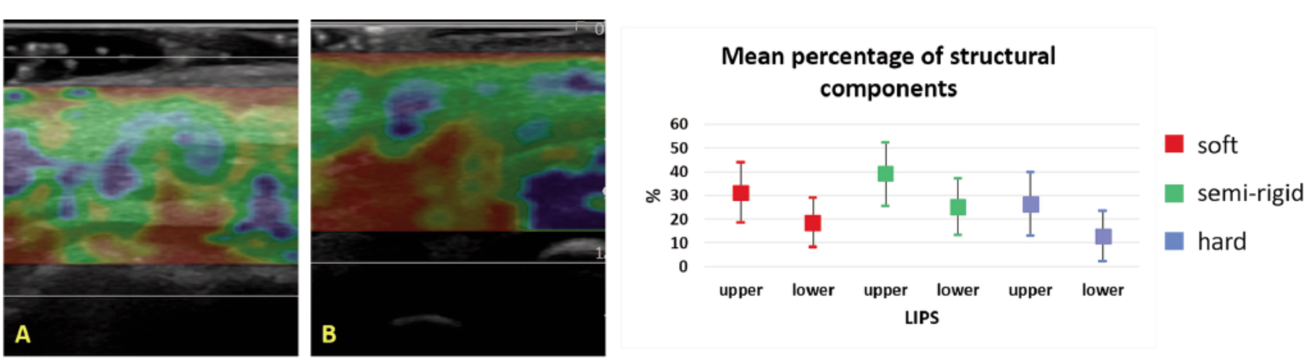


**Figure 9**. Elastosonography analysis of the upper (**A**) and lower (**B**) lip in a 42-year-old subject. In the structural pattern of both areas it is evident how the semi-rigid components, made up of collagen fibers, are dominant and contribute to determining the fibrous nature of the areas themselves. Although the structural pattern is similar, there is still a percentage difference in the number of components present in the two areas, with higher percentages for the upper lip, underlining that, despite being similar, these areas are distinct areas of the face.

sent in the hypodermic tissue. Like all other types of facial wrinkles, these wrinkles also have a dubious genesis, which includes muscle movement, loss of volume of adipose tissue, and sliding of fat compartments.[12,13] Furthermore, there is wide variability among subjects. Let's consider for example that in some subjects ptosis of the eyebrows occurs with age, especially in the lateral area, in others the opposite happens and the eyebrows rise, while in some people no change is noted in the position of the eyebrows.[14] Variations in the eyebrows position are related to gender and race,[14] and can reasonably be strongly attributed to the volumetric and positional changes in fat compartments, bone resorption, and gravity. As regards horizontal wrinkles, their presence in both male and female subjects and of different ethnic groups suggests their greater dependence on the movement of the frontal muscles, according to the mechanism well described by Kondoh *et al.*,[11] but also to the loss of volume of adipose tissue. In fact, the subjects considered in the present study did not show a decrease, but an increase in adipose tissue, and no wrinkles were evident on the forehead.

## Zygomatic region

The superficial subcutis of this region was characterized by a dense intertwining of small connective shoots with a wavy pattern and an organization that can be defined as an "accordion" (Figures 5 and 6). This organization is fundamental for cheek movement, and facial expressions, and differs completely from the structure of the deep adipose tissue compartment. The different structure of the superficial and deep fat compartments can be justified in the functional role of the compartments themselves. While the superficial

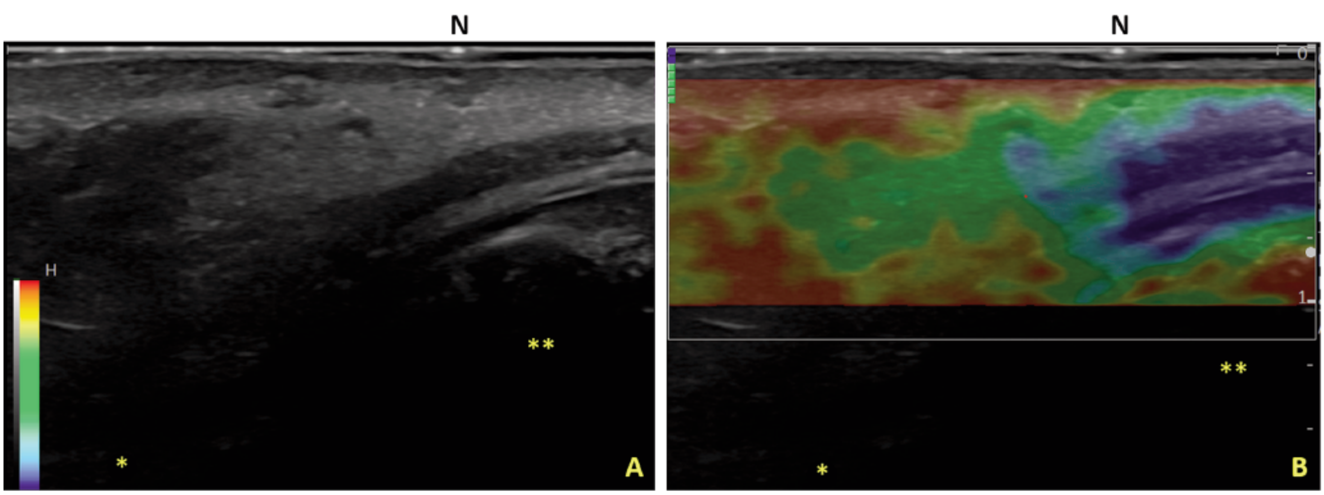


**Figure 10**. Ultrasonography in a 31-year-old subject. The nasolabial fold divides the buccal area from the cheek, two areas that have a completely different structure. **A**) Ultrasound analysis highlights that while the lip is compact with a dense network of connective tissue, and scant adipose tissue, a superior adipose compartment, the nasolabial compartment, is superficially identified in the cheek. **B**) The elastosonography analysis emphasizes the structural difference, which results in a difference in the elastic properties of the two areas. N, nasolabial fold; *cheek; **buccal cavity.

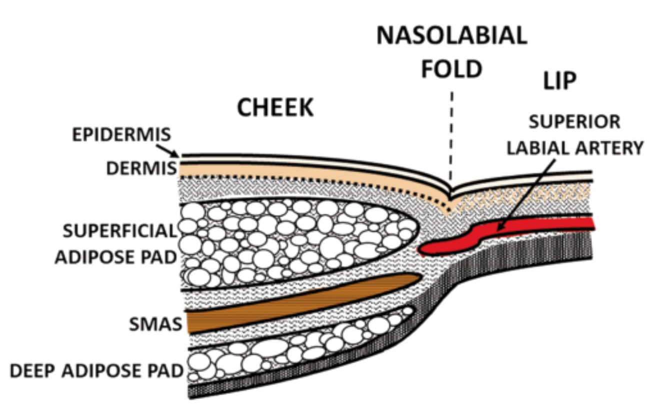


**Figure 11**. Schematization of the structures of the epidermis, dermis and hypodermis at the level of the nasolabial line. The connective structure appeared different at different depths. The structure of the upper lip was described in Figure 7. At the level of the cheek, superficially the connective fibers were oriented parallel to the skin. A more elastic structure was noted between the superficial and deep fat compartments, a connecting structure between different layers. In depth, the connective fibers had an orthogonal arrangement, anchoring the fat pads to the underlying structures.

ones contribute significantly to facial expression, chewing and speech, and therefore must have high mobility, the deep, almost static compartments seem to perform the fundamental task of anchoring the entire upper anatomic structure to the bone. A clear example of this difference in motility has already been demonstrated, using ultrasound, by Schelke et al.[15] They proved that the movement of the superficial layer determines the mimicry of the smile, unlike the deep adipose layer, which remains practically immobile, during this expression.

The mobility of the superficial structures of this area, fundamental for daily life, however, entails greater wear of all the tissues present, making them more subject to the effects of aging. In fact, in this area both effects of gravity and decay, loss of volume, displacement of compartments, as well as greater laxity of the ligaments and weakening of the muscle fibers were observed.[1,2,4,13,16,17]

## Upper and lower lip regions

The structure of the labial regions appears as a single ribbon (Figures 7 and 8), that stretches laterally and medially during movements related to facial expression and language, while movements in the frontal plane are absent. The type of aging observed in these areas seems to be mainly connected to alterations of the extracellular matrix[1] and occurs orthogonally to the motor plane, with the formation of barcode wrinkles in the upper lip region. The alterations of the extracellular matrix manifest themselves both in terms of fragmentation of the collagen matrix with collapse of the fibroblasts,[18] and in terms of reduction of the elastic components.[19]

The alteration of the muscular structure of the orbicularis muscle can also contribute to the formation of these wrinkles,[13] and there seems to be a directly proportional relationship between the number of wrinkles and the amount of movement that the lip makes. This may be why these wrinkles are more noticeable in smokers,[20] who perform more movements than normal chewing and speaking movements compared to non-smokers.

The fact that wrinkles do not form in all people and that there are gender and ethnic differences in the rates of their appearance,[20,21] despite all people eating and talking, would seem to indicate that other factors contribute to aging in addition to use. Considering the observed structured, it is believed that a correlation with volumetric changes, compartmental slips and changes in bone structure, primary factors of aging in other areas of the face, can be excluded. Undoubtedly, more in-depth studies are needed.

## Nasolabial fold

The nasolabial line is probably the most studied facial line because its depression is one of the most obvious and common signs of age. It is also one of the most complex lines of the face, as it clearly separates extremely different compartments (Figure 10).[22]

The dynamics by which this line becomes more evident during aging have also been the subject of numerous studies.[2,15-17,23,24] Rohrich *et al*.[2] described the process of cheek ptosis in detail, while Gierloff *et al*.[23] and Stuzin *et al*.[17] characterized the volumetric dynamics of the adipose compartments and the consequent rebalancing movements of the same. Indeed, citing Beer *et al*.,[24] "the nasolabial fold is the product of a complex interaction between local tissues" and other authors have described these dynamics.[16] It should be emphasized that this is an extremely wear-prone line, as it is affected both of the movements of the mouth and those of the cheek and jaw.[15] Finally, given that in the supine position it disappears in 86.1% of people,[25] it is appropriate to highlight the role that gravity and sagging play in its severity. This is compatible with the absence of differences observed in the twenty-four subjects of this study, in whom there was no structural difference, and in whom the flattening of the nasolabial fold in the semi-reclined position was evident.

In the considerations made on the dynamics of aging, sun exposure was not considered, as it is an external cause that varies from person to person.[12] It is clear that, with aging, different areas of the face behave differently, but in the same area, such as the cheek, even the superficial and deep fat compartments, different in volume and structure, behave differently. Therefore, to understand aging process, it is necessary to study both the individual aging of the different structures in each area of the face, the interrelationships between different structures of the same area, and the interrelationships between the different areas.

What does this study add to current knowledge and practices? This study demonstrates that the differences in the areas of the face most treated with fillers concern fat deposits, muscles, but also all the components of the extracellular matrix, collagen *in primis*. Therefore, when intervening, for instance, with a filler, one must choose whether to operate only for aesthetic purposes, filling spaces and smoothing out wrinkles, or also for regenerative purposes. In the latter case it is essential to resort to non-invasive analysis techniques that allow the injector to understand if and what are the alterations of the anatomical structure he/she wants to treat, and which are the tissues with which the filler must mainly interact, to resolve the specific problems.

Specifically, this anatomical study, performed before the injection of fillers in the 24 subjects, oriented the choice of the injector towards different high and low molecular weight products. The former, in fact, have a greater filling effect, slow absorption and, therefore, a longer permanence in the tissues. The latter do not have a filling effect, absorption is rapid and their action is above all bio-stimulation.

The results of the present study seem to demonstrate that there are constant structural and age-related differences between different segments of the face. In reality, given that the number of subjects considered in this study is limited, further studies are needed to define a true aging model. Just think of the data collected for the forehead which seem to conflict with what is known from the literature. However, this discrepancy makes us understand the enormous heterogeneity of people's forms of aging, and that models different from the standards are possible. Therefore, for each person, an in-depth study of the facial anatomy before a treatment is essential to optimize the choice, and application of the treatment itself. When it is not possible to analyze a biopsy sample of the area to be treated, to understand the characteristics and dynamics of this area, ultrasound with a 24 MHz probe seems to be the most suitable method. To evaluate the structural components, elastosonography could be a very valid method. Modern ultrasound techniques can provide similar histological information.

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