CASE REPORTS

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Anatomopathological findings in scars: comparative study between different specimens

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Abstract

In spite of the remarkable progress science and medicine have experienced, many facts concerning healing processes and pathological scars are still unknown or incompletely explained. This paper is part of a larger study (research for a PhD thesis) concerning new approaches in the prevention and treatment of pathological post-burn scars. We present and analyze the cases of some patients who developed abnormal scars in order to understand and point out the characteristics, that different types of pathological scars have in common and how we can differentiate them. Knowing what issue to address is the key to any successful therapy. Thus, the information we obtained will help us in applying more appropriate and efficient methods of treatment and in our further research: comparing the efficiency of newer therapies to that of older ones.

Keywords: pathological scar, hypertrophic, keloid, retractile.

Introduction

Pathological scars have been widely seen from the oldest times. The oldest historical mentioning of abnormal scars was probably the Smith' papyrus, written around ~1700 BC (before Christ). Only very late, in the 1960' and the 70', the pathological scars were classified and the criteria and definitions for keloid and hypertrophic scars were clearly stated. We have nowadays an advantage due to the advances that have been made in research concerning imagistic, biomolecular and biochemical fields, which provide us with minute information about the intracellular processes and the histological changes that appear during healing and scar formation. There are still a lot of things that are not known yet, a lot of factors that have not been identified or processes that are so complex or have not been studied enough, so the information about keloid and hypertrophic scars contains many gaps that are to be filled in the years to come. Still, there are no established protocols and standards in the treatment of abnormal scars, but merely some general guidelines [1–6].

Therefore, "hypertrophic scars and keloids result from an abnormal fibrous wound healing process in which tissue repair and regeneration-regulating mechanism control is lost. These abnormal fibrous growths present a major therapeutic dilemma and challenge to the plastic surgeon because they are disfiguring and frequently recur" [6]. They are very noticeable, conspicuous especially in exposed areas and thus they are not only disturbing because of the symptoms they may cause (like pain, pruritus, limiting of the movement range), but raise also esthetic problems [1, 5, 6].

Having this in mind, the purpose of this article is to present the prospective study we performed on various types of scars, of different etiologies. We tried to compare the pathological images and to draw some conclusions, concerning things that all of these scars have in common, in spite of their origin and to point out the particularities for each one.

Case reports

We selected some cases that we will present and compare to normal findings in such situations. We will also point out the things, that the scars from different patients had in common and the facts, that are different form one case to another.

The starting point for this paper is the following information that we already know about scars.

From a gross viewpoint, the keloid and hypertrophic scars look macroscopically very similar. No clear differentiation can be made, unless one knows how big the lesion was initially and then sees the scar in its evolution process. To sum up the main characteristics are pointed out in Table 1.

Now, the symptoms that accompany these scars are also very unspecific, being quite the same in both cases. Still, it is very important to know exactly whether a scar is a keloid or a hypertrophic scar, as the treatment plan depends on the type of scar. It is important to keep in mind too, the fact that keloids resemble tumors, as there will appear an abnormal growth process that extends into healthy tissue, over the boundaries of the initial wound.

How do we make the difference between keloid and hypertrophic scars? In all cases, the morphological-patho-

logical examination is the one that sets the exact diagnosis. There are some key-features that were analyzed, by many studies concerning this issue, using the light microscopy, the electronic microscopy and immunohistochemistry: the disposition and organization of the collagen and the presence of α-SMA (alpha-smooth muscle actin) in myofibroblasts.

Table 1 – ***Comparison between hypertrophic and keloid scars (from Wolfram et al., 2009 [6])***

Hypertrophic scars	Keloids
Develop soon after surgery.	May develop months after the trauma.
Usually improve with time.	Rarely improve with time.
Remain within the confines of the wound.	Spread outside the boundaries of the initial lesion.
Occur when scar cross joints or skin creases at a right angle.	Occur predominantly on the ear lobe, shoulders, sternal notch, rarely across joints.
Improve with appropriate surgery.	Are often worsened by surgery.
Frequent incidence.	Rare incidence.
Have no association with skin color.	Associated with dark skin color.

Case No. 1

S.M.G., male, 20-year-old, had 2nd and 3rd degree burns (by flame) on neck, trunk, face and upper limbs, that were treated by excision and grafting or conservatively, by applying ointments daily. After approximately two years after the event, he has pathological scars and retractile ones on his neck and jawline (Figures 1 and 2).

We excised a fragment of scar (0.8/1/0.8 cm) from the inferior 1/3 of the left cheek with a border of 1–2 mm of healthy tissue. We injected the rest of the scars, situated by the jawline with corticosteroids (Kenalog). We plan to do some "Z-plasty" procedures, in order to release the retractile scars from the neck, after the scars have matured and stabilized.

Macroscopically, the tissue fragment had increased consistency and diminished elasticity; its color (reddish) was different from the surrounding skin, as noticeable in Figure 2. Its surface was very smooth and shiny and hair lacked in the described area. The patient experienced sometimes itch and pain or altered sensitivity in the area of all these scars. However, they did not have a functional impact; they did not restrain movement and daily activities. The esthetic and psychological impact however are noticeable, as the patient does not want to talk about the accident and recovery period and as he said that he experiences great discomfort even after he is healed, due to the pain. He also always uses a scarf or high collar to cover up, to avoid any questions and inquisitive looks from people.

Microscopically, the fragment was examined after using the classical Hematoxylin–Eosin (HE) staining (Figures 3–5).

One can notice the presence of a distinct lesion that is situated in the central (mid) area of the dermis, which contains an abundant amount of collagen and fibroblasts (Figure 3). The superficial dermis is apparently normal from a histological point of view (Figure 5). It is important to observe numerous capillaries in the depth of the lesion (Figure 4). No structures belonging to appendices of skin can be identified. Therefore, the diagnosis is probably hypertrophic scar.

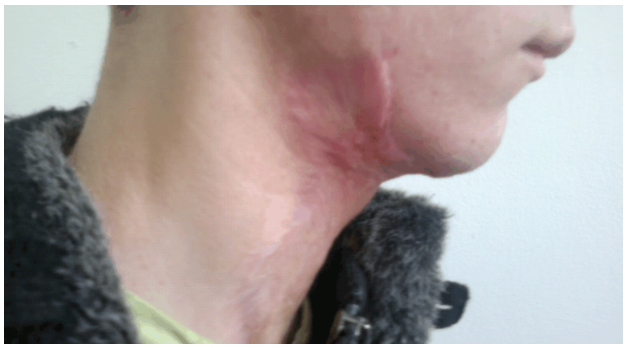

Figure 1 – ***Post-burn scars (hypertrophic) in a 20-year-old patient (Case No. 1).***

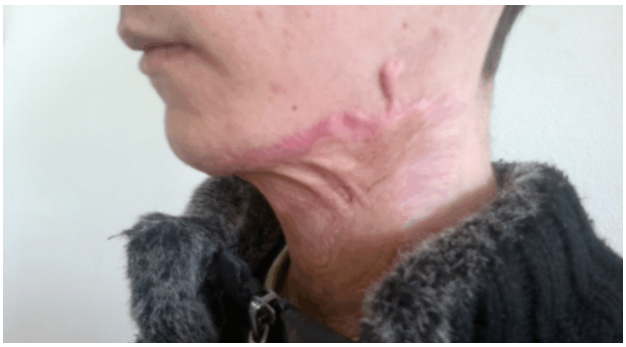

Figure 2 – ***Hypertrophic post-burn scars. The vertical scar situated on the inferior third of the cheek was excised and analyzed.***

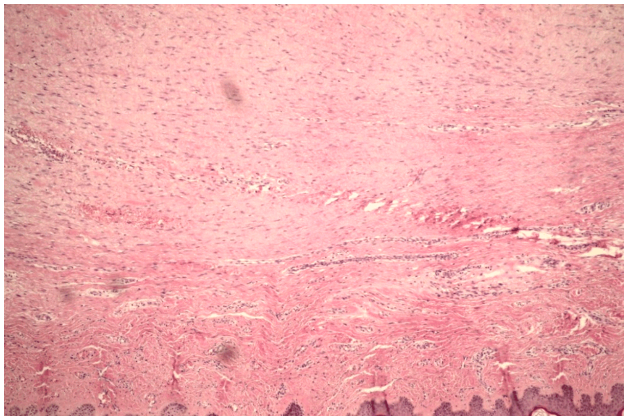

Figure 3 – ***Abundant amount of collagen and fibroblasts in the mid-dermis. HE staining, ×10.***

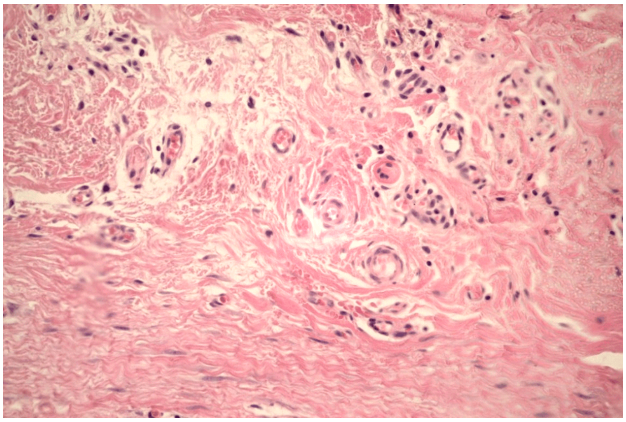

Figure 4 – ***Numerous capillaries in the depth of the scar tissue. HE staining, ×40.***

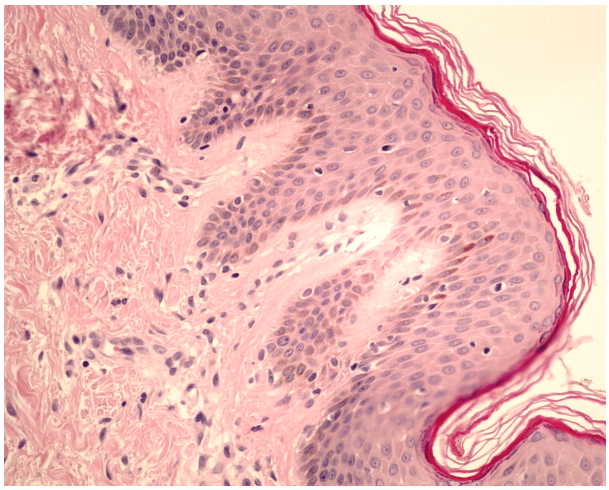

Figure 5 – ***Thickened epidermis, apparently normal superficial dermis, numerous capillaries in the deeper dermis. HE staining, ×40.***

Case No. 2

B.G.E., female, 30-year-old, had two small cutaneous tumors (that were diagnosed as nevi) removed from the inferior pole of her right breast; after approximately two years after surgery, the patient's scars had turned in two keloids (Figure 6). We excised the two lesions and afterwards we started early injection of the newly resulted postoperative scars with corticosteroids (Kenacort).

Macroscopically, the fragments had the following dimensions 4–5/1/0.5 cm and 3–4/1.5/0.5 cm and included margins of healthy tissue. They were a lot larger than the initial excision margins. The patient stated that they grew noticeably from a day to another, but they did not hurt or itch. In fact, her sensitivity in the area was diminished.

The skin in these fragments was inconstantly thickened and smooth. The scars had increased consistency and they were lacking hair.

Microscopically, we notice dermal sclerosis (Figure 7), very thick collagen bundles in the superficial dermis, perivascular agglomeration of collagen and inflammatory infiltrate (Figure 8). To be more specific: the cutaneous fragment exhibited an important amount of collagen that prevailed in the superficial layer of the dermis, associated with a chronic subepithelial perivascular lympho-plasmocytary inflammatory process. There is also a substantial decrease in the number of cutaneous appendices. The deep layer has normal histology. The epidermis is orthokeratotic. The described features suggest two active keloids.

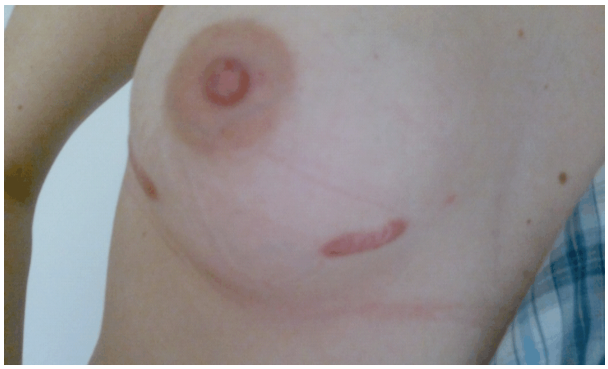

Figure 6 – ***Postoperative keloid scars (Case No. 2) in 30-year-old woman. Both scars were excise and analyzed from anatomopathological point of view.***

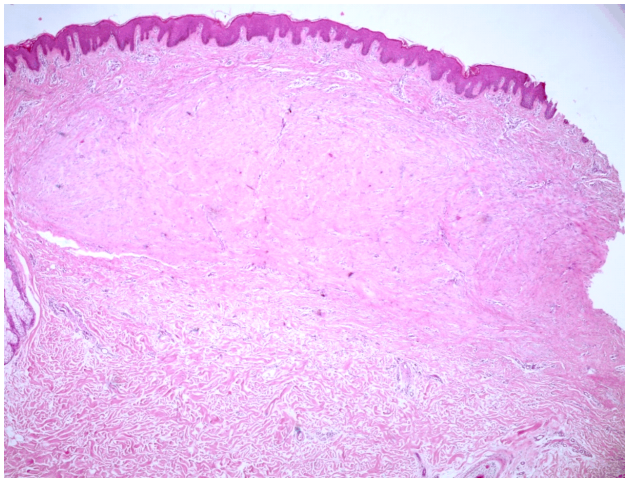

Figure 7 – ***Dermal sclerosis. HE staining, magnifying glass.***

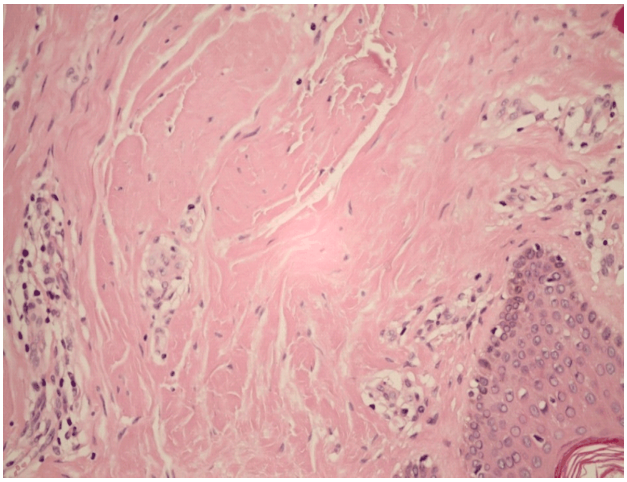

Figure 8 – ***Thick collagen bundles in the dermis' superficial layer. Blood vessels and inflammatory infiltrate. HE staining, ×40.***

Case No. 3

S.I., male, 26-year-old, was submitted and treated for 2nd and 3rd degree burns (electrical flame) on the cephalic extremity, anterior and posterior trunk and both upper limbs, about six months ago. Some lesions healed, some required 2–3 sessions of grafting. Overall, the patient's evolution was a good one, the scars are acceptable, but he developed, as expected some retractile ones, the tightest being at the right humeral joint (Figures 9 and 10).

He also recovered well functionally, after such extensive burns. He only has limited motion range in the above-mentioned joint, being incapable of lifting the arm more than 45–50^0 above the trunk. The other joints in the upper limbs are fully functional, after he has undergone several sessions of physiotherapy and kinesiotherapy.

We excised a fragment (1/0.5/0.4 cm) of this retractile scar, in order to study it in detail.

Macroscopically (Figure 11), we notice that the scar follows the anterior border of the humeral joint and

restricts the lifting of the arm, no matter if anterior, lateral or posterior and the circumduction. Its color resembles the surrounding tissues. Its surface is extremely smooth and translucent, very sensitive during daily tasks. The patient experiences bleeding and pain frequently.

Microscopically (Figure 12), one can observe the following pathological findings in the examined tissue (that is similar to the skin): microulcerations and bleeding, exocytosis and parakeratosis. Using HE staining, we found that the dermis is housing a flourishing inflammatory process, heralding numerous polymorphonuclear cells (neutrophils and eosinophils – in various developmental and degradation stages), macrophages, including giant "foreign-body" cells and lymphocytes. At this same level, we notice the presence of neovascularization that stands as proof for the existence of an inflammation-reparation process of questionable effectiveness (Figure 13).

Except for the ulcerated areas, there cannot be identified areas of basal membrane discontinuity (Figure 14). The inflammatory process is accompanied by edema, hemorrhage and tissue necrosis (Figures 15 and 16).

Discussion

We have to point out that the key of a correct and effective treatment is to establish from the very beginning with what kind of scar you are dealing, as Mustoe *et al.* explained it too, in one of the few guides that are available and were renewed in 2013 concerning scar management [4].

After analyzing these different types of scars, we could understand the evolution of such lesions better and assess what exactly is going wrong.

As we said before, in the "Case reports" section, two key features are essential in establishing whether a scar is a hyertrophic or a keloid one.

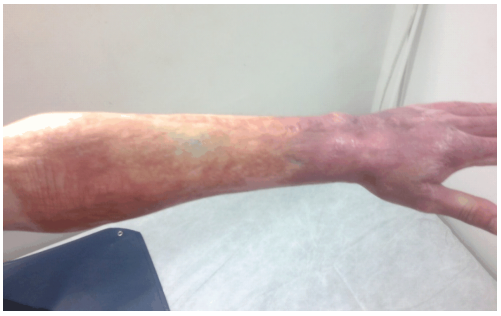

Figure 9 – ***Post-burn scars in 26-year-old patient (Case No. 3). These scars are considered to be pretty satisfying from esthetical and functional points of view.***

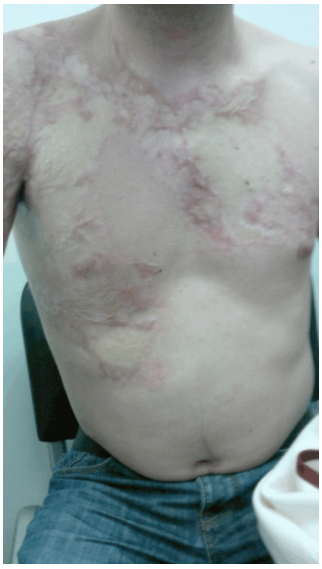

Figure 10 – ***Post-burn scars in the same patient (Case No. 3), there are areas that were treated by excision and grafting – this is the cause of the depigmented areas.***

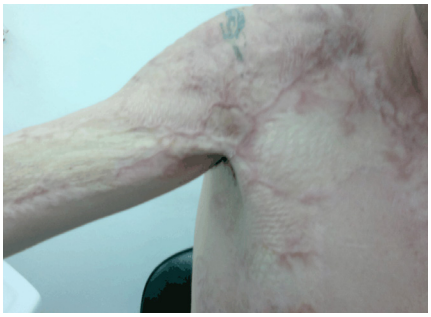

Figure 11 – ***Retractile scar following the anterior axillary line. The patient cannot raise his arm above the photographed angle. A fragment from this area was excised and analyzed.***

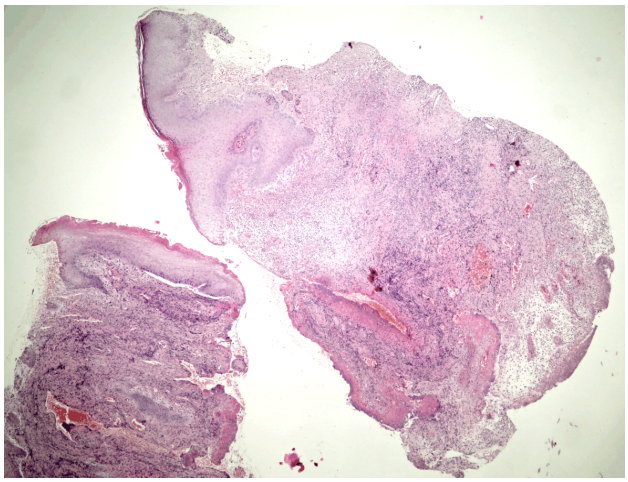

Figure 12 – ***"Bird's eye view" of a section through a fragment of a retractile scar. HE staining, magnifying glass.***

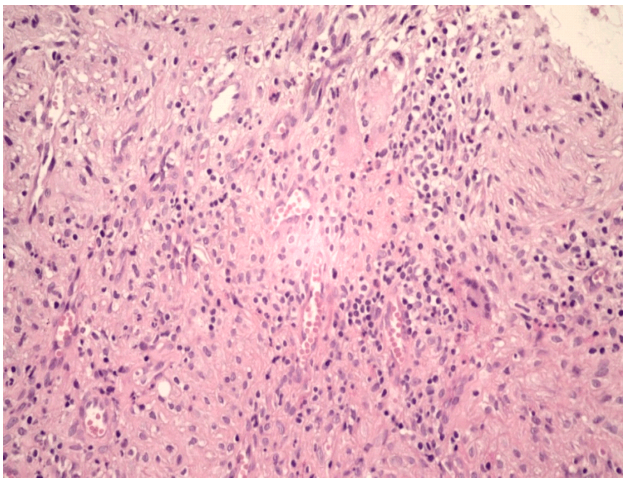

Figure 13 – ***Giant multinuclear cells. HE staining, ×40.***

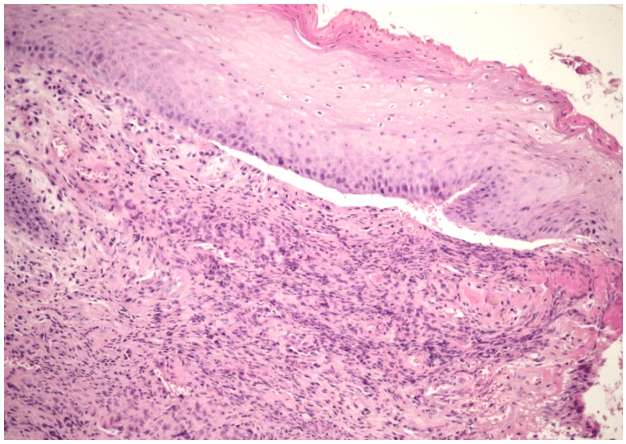

Figure 14 – ***Dermo-epidermal dehiscence. HE staining, ×20.***

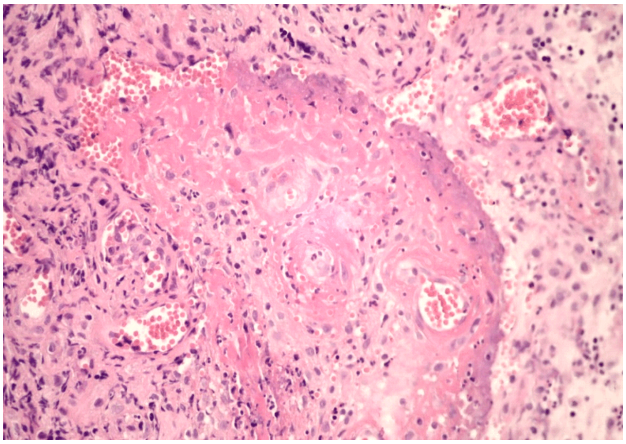

Figure 15 – ***Inflammation process associated with necrosis. HE staining, ×40.***

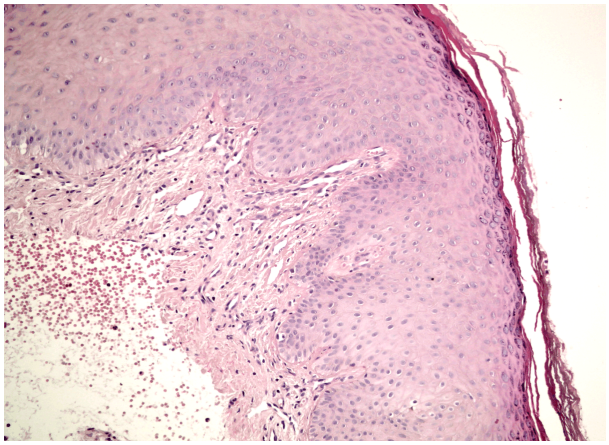

Figure 16 – ***Thickened epidermis, due to acanthosis; numerous newly formed subepithelial blood vessels. Edema and hemorrhage in the mid-third of the dermis. HE staining, ×20.***

Disposition and organization of collagen

Using the electronic microscopy it has been noticed that keloids contain bundles of big, thick collagen fibers, made up by many, intimately united fibrils and fibroblasts, around which there is an amorphous extracellular substance. "Electron microscopic examination supports the … differences in collagen organization and in fibroblastic features and shows the presence of an amorphous extracellular material surrounding fibroblastic cells in keloid" [5]. The keloid scars are made up of a huge amount of completely disorganized collagen (mainly type I and II) and the extracellular matter holds a great amount of mucopolysaccharides. In opposition to this image, in hypertrophic scars one can observe nodular structures that contain fibroblasts, small blood vessels and fine collagen fibers, randomly arranged. Thus, the thick bundles are characteristic for keloid scars. The hypertrophic scars' nodular structures resemble the ones that can be found in Dupuytren's disease, being oriented parallel to the surface of the skin and having the same trajectory as tension lines of the skin [6–8]. The main collagen found in them is type III. There are cases in which the nodular structures described above where found coexisting with large bundles of collagen fibers, thus being suspected that the scar is a mixture between keloid and hypertrophic. In keloids, the collagen tends to occupy the whole reticular dermis, while in hypertrophic ones it is found only in the more superficial layers of the reticular dermis, this being part of the explanation why keloid scars spread in tumoral fashion, over the borders of the initial wound. Another part of this explanation has to do with the more abundant inflammatory phase and process that takes place in keloids: there can be noticed an inflammatory infiltrate, that is a stimulus for the abnormal synthesis of extracellular matrix by the fibroblasts (in a keloid scar the amount of collagen synthesized is about 20 times greater than in a normal scar). While the inflammation decreases in hypertrophic scars (which explains why some hypertrophic scars regress with time), in keloid scars it keeps happening [6, 7–9].

Presence of α-SMA in myofibroblasts

In hypertrophic scars, there were found myofibroblasts that express the protein α-SMA on their surface. This protein was studied too and the conclusion was that it is an isoform that only appears in the walls of blood vessels, which explains why hypertrophic scars taper off in time, while keloids never do [6, 5].

Apoptosis

This is a central factor in determining the type of resulting scar. Once the epithelization process and the synthesis of collagen have set on, the cellularity in a physiological scar starts to decrease progressively. In tissues that are healing normally, the myofibroblasts appear on a transitory basis, and disappear completely. A hypertrophic scar has the following characteristics: it is hypercellular for a much longer time than a physiological scar. It starts resembling normal tissue, once its maturation and remodeling have begun. In a physiological healing process, the myofibroblasts' apoptosis starts at about 12 days after the injury, having a peak at about 20 days; in hypertrophic scars, it reaches a peak only at about 19–30 months after the injury [6, 10].

Comparing the normal skin to the scar tissue, we noticed:

- in hypertrophic scars and keloids, the connective tissue is found in greater amounts than normal, accompanied by numerous newly formed blood vessels. The cellular concentration is also much higher than in the dermis of normal skin/physiological scar tissue;

▪ sometimes, the epidermis is thicker in hypertrophic scars and keloids;

▪ the skin appendices (sebaceous glands, hair follicles and sweat glands) are absent (or in extremely small amounts) in all kinds of pathological scars;

▪ the retractile scars have a completely disorganized architecture, which explains partly why they are so thick and functionally impairing. Due to the fragile epidermis, their superficial layers break down often, thus resulting in ulcerated areas.

Ehrlich *et al.* observed abnormally large amounts of collagen in the entire dermis in keloids, amounts that were absent in hypertrophic scars [5] or at least concentrated in the deeper dermal layers. In regular scar tissue, collagen is found in the mid-dermis.

Superficial newly formed blood vessels are present in keloid scars. These can be visualized through the transparence of the skin and resemble telangiectasia. A process of neovascularization is encountered in hypertrophic scars too, but the small blood vessels are situated deeper, around the nodular structures, that are characteristic for this type of scar tissue. Both types of pathological scars are thus hypoxic tissues [8].

Regular scars have less cellularity, virtually extremely rare cells, compared to any pathological scar. [9]. It has been proved that the ratio collagen type I/collagen type III is increased in post-burn scars and that the orientation of the collagen does not assume the "basket weave appearance" seen in normal skin [11].

The epidermis was always flattened in hypertrophic scars, but not frequently in keloids. Hyperkeratosis and hypergranulosis appeared in all types of scars, even in normal ones [9].

An imbalance in the inflammatory cells subpopulations, more exactly in the subtypes of lymphocytes – CD4(+): CD8(+)(Th:Ts) – seems to be one of the causes of abnormal healing and pathological scars in humans. Even more, one of the new hypotheses is that people, who develop abnormal scars, especially the ones predisposed to keloids, have alterations in their immune system [8, 12, 13].

There are conflicting data provided by different research groups concerning the pathological scars, but we think, that the facts that we pointed out, in correlation with clinical data can help us in setting the correct diagnosis for a particular case, which will lead to applying the adequate treatment plan.

Conclusions

There have been many studies performed concerning anatomopathological imaging of scar tissue, so through this paper we did not discover or invent anything, but we merely tried to approach different types of scars to study them ourselves, in order to fathom the processes, that are going on. This small study is part of a larger doctoral one, presenting some new methods of prevention and treatment of pathological scars, in comparison to the older, already known ones. The information we gathered will be of great use and help us in our further work, as the key to recommending a treatment method is knowing what it has to address, what to treat.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Sood R, Achauer BM. Achauer and Sood's burn surgery: reconstruction and rehabilitation. Elsevier–Saunders, Philadelphia, 2006, 3–36.

[2] Florescu IP. Contribuţii la tratamentul cicatricilor hipertrofice şi cheloide. Chapters 1 and 2. PhD Thesis (scientific supervisor: Prof.Dr. Vasile Fufezan), I.M.F. Timişoara, 1996.

[3] Chiotan N, Matusz P, Florescu I. Cicatrizarea – biologie clinică – tratament. Chapter 1. Ed. Naţional, Bucureşti, 1999.

[4] Mustoe TA, Cooter RD, Gold MH, Hobbs FD, Ramelet AA, Shakespeare PG, Stella M, Téot L, Wood FM, Ziegler UE; International Advisory Panel on Scar Management. International clinical recommendations on scar management. Plast Reconstr Surg, 2002, 110(2):560–571.

[5] Ehrlich HP, Desmoulière A, Diegelmann RF, Cohen IK, Compton CC, Garner WL, Kapanci Y, Gabbiani G. Morphological and immunochemical differences between keloid and hypertophic scar. Am J Pathol, 1994, 145(1):105–113.

[6] Wolfram D, Tzankov A, Pülzl P, Piza-Katzer H. Hypertrophic scars and keloids – a review of their pathophysiology, risk factors, and therapeutic management. Dermatol Surg, 2009, 35(2):171–181.

[7] Gauglitz GG, Korting HC, Pavicic T, Ruzicka T, Jeschke MG. Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. Mol Med, 2011, 17(1–2):113–125.

[8] Moshref SS, Mufti ST. Keloid and hypertrophic scars: comparative histopathological and immunohistochemical study. JKAU Med Sci, 2010, 17(3):3–22.

[9] Gauglitz GG, Pavicic T. Emerging strategies for the prevention and therapy of excessive scars. MMW Fortschr Med, 2012, 154(15):55–58.

[10] Sternberg SS, Mills SE, Carter D. Sternberg's diagnostic surgical pathology. 4th edition, Lippincott–Williams & Wilkins, 2004, 36–37, 1040–1041.

[11] Rawlins JM, Lam WL, Karoo RO, Naylor IL, Sharpe DT. Quantifying collagen type in mature burn scars: a novel approach using histology and digital image analysis. J Burn Care Res, 2006, 27(1):60–65.

[12] Boyce DE, Ciampolini J, Ruge F, Murison MS, Harding KG. Inflammatory-cell subpopulations in keloid scars. Br J Plast Surg, 2001, 54(6):511–516.

[13] Florescu I, Bratu T, Matusz P, Chiotan N, Luculescu P. Tratamentul cicatricilor cutanate. Ed. Mirton, Timişoara, 2000, 1–40.

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